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Review Article

The role of assisted reproductive biotechnology (ARTs) in conservation biology

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ABSTRACT

Assisted reproductive biotechnology (ART) has emerged as a promising tool in the field of conservation biology, offering new opportunities for the preservation and management of endangered and wild species. This review article explores the role of ART in conservation biology, focusing on its applications, benefits, and challenges. ART encompasses various techniques such as *in vitro* fertilization, gamete cryopreservation, and embryo transfer, which can be adapted to address issues related to low fertility, genetic diversity, and reproductive disorders in endangered species. The use of ART in conservation efforts allows to produce genetically diverse offspring, the rescue of endangered gametes, and the establishment of captive breeding programs. However, several challenges persist, including the ethical considerations surrounding the use of ART, the high costs involved, and the potential risks to individual welfare and long-term species viability. Therefore, the effective integration of ART in conservation biology requires careful planning, collaboration between scientists and conservation practitioners, and the development of comprehensive strategies that consider both short-term and long-term conservation goals. Ultimately, ART has the potential to play a crucial role in the conservation of endangered species, providing a valuable complement to traditional conservation approaches and contributing to the preservation of biodiversity for future generations

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Introduction

The use of assisted reproductive biotechnology (ART) in conservation biology has garnered significance over the last couple of years. The field of ART has expanded considerably in the span of last several decades, providing new opportunities for conservation biologists to address reproductive challenges faced by endangered species (Comizzoli 2015). According to a recent review by Mocé et al. (2021), ART has been successfully used in a variety of conservation scenarios, including captive breeding, reintroduction programs, rescue of endangered species from extinction, and creation of genetically diverse populations (Mocé et al., 2021). ART can help to overcome obstacles such as poor semen quality, low sperm production, infertility, and small population size, and can help to maintain genetic diversity and fitness in populations of threatened species (Swanson 2023).

Reproductive biotechnologies have emerged to reduce generational intervals and facilitate the spread of genetic material within animal breeding populations (Kaya et al. 2018). To realize this objective, a series of reproductive technologies have been progressively developed. These include artificial insemination (AI), embryo transfer (ET*), in vitro* fertilization (IVF) with manipulation of fertilization and embryo production, as well as cloning for transgenesis applications (Mukherjee et al. 2023). Additionally, there is a growing trend of commercialization surrounding these technologies, including sperm separation techniques such as sex-sorting, which involves the selection of spermatozoa based on chromosomal sex (Rodriguez-Martinez 2012).

Despite the remarkable progress achieved in the discipline of reproductive physiology in past few years, the challenge of infertility persists, characterized by low conception rates and high embryonic mortality rates (Thoma et al. 2021). To meet future demands and sustain agricultural production, it is crucial to leverage emerging technologies, particularly modern reproductive biotechnologies. Reproductive techniques such as estrus synchronization, superovulation, non-surgical embryo collection and transfer, cryopreservation of embryos, oocyte retrieval from live animals, fertilization, development of embryo, *in vitro* maturation, and cloning have been developed (Poonia et al. 2023). However, their potential to enhance animal production has been hampered by the limited availability of cost-effective embryos from high-quality animals. Nevertheless, recent scientific advancements in assisted reproductive techniques offer promising opportunities to revolutionize the animal world by enabling manipulation of reproductive processes (Verma et al. 2012).

The primary objective of species protection is to conserve biodiversity, as the elimination of even a single species can have profound effects on the overall ecosystem dynamics (Chase et al. 2020) (Henson 1992). To preserve the natural adaptation of animals, in situ conservation strategies are implemented to maintain live populations within their native environments (Mestanza-Ramón et al. 2020). However, these efforts may not always be adequate for the successful expansion of small populations and ensuring sufficient genetic variation is maintained (Andrabi and Maxwell 2007). This review aims to provide an overview about different assisted reproductive techniques and their uses in conservation of biology, and their application in conservation in wildlife and endangered species.

1. Evolution of Assisted Reproductive Technologies:

Assisted reproductive technologies (ARTs) have evolved significantly since the early 20th century. The first experiment with artificial insemination began in the 1930s, laying the groundwork for future developments (Gurtler 2013). The breakthrough moment came in 1978 with the birth of Louise Brown, the first baby conceived through *in vitro* fertilization (IVF) (Fishel 2019). Since then, ARTs have continued to advance, introducing techniques like embryo transfer, intracytoplasmic sperm (ICSI), and preimplantation genetic diagnosis (PGD), revolutionizing fertility treatments.

It highlights the key developments, their significance, and the broader trends that have shaped the field over time.

Advancements in ARTs for wild animals

This paper will delve into the various aspects of reproductive technology, including *in vitro* fertilization, separated semen, and translocation of nucleus or cloning. Every single one of these techniques will be examined thoroughly within the context of this discussion.

Overview of ARTs

In vitro fertilization and embryo transfer

During the mid-1990s, several commercial IVF laboratories emerged in Canada, the United States, and Europe, specifically in Germany, Italy, France, and Holland (Gerrits 2021). Over time, additional laboratories were established in South America, such as Brazil and Argentina, as well as in Oceania, including Australia and New Zealand. The introduction of transvaginal ovum pick-up guided by ultrasonography (OPU) played an important part in facilitating the use of IVF in live females (Bols and Stout 2018). This technique enabled the retrieval of eggs from females who may face challenges in producing offspring through conventional reproductive methods. Initially, the primary objective of commercialized IVF was to acquire viable embryos from female subjects who may have reproductive difficulties using traditional techniques (Faber and Ferré 2004). In addition to facilitating the production of animals with high genetic merit, *in vitro* production technologies also serve as a valuable source of embryos for various emerging biotechnologies (Ferré et al. 2020). These include techniques such as embryo sexing, cloning, nuclear transfer, and transgenesis. The utilization of *in vitro* production technologies not only contributes to the breeding of superior animals but also opens opportunities for advancements in other biotechnological applications (Verma et al. 2012).

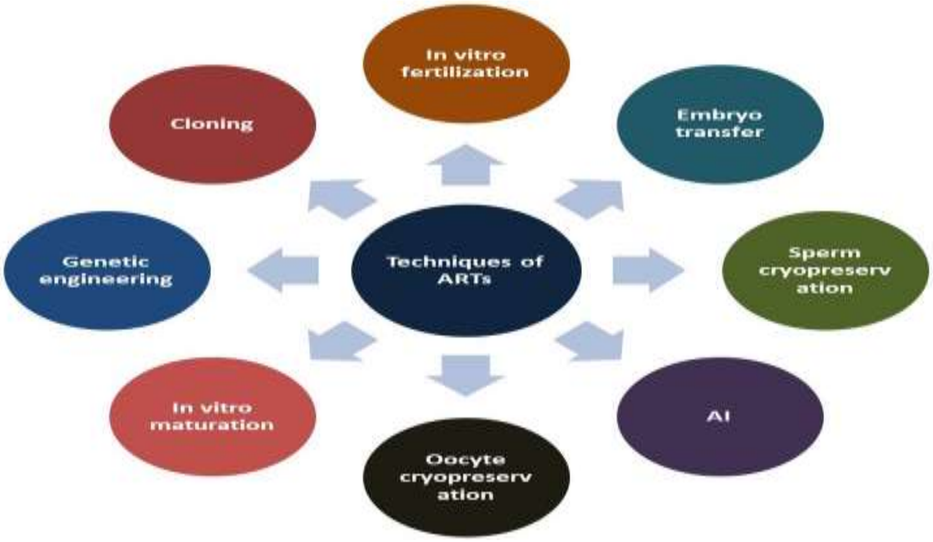

Fig. 1: The different techniques used in assisted reproductive techniques for the conservation of biology, AI, and artificial insemination.

Interspecies embryo transfer (ET) continues to receive limited attention, and there is a lack of comprehensive knowledge regarding the embryonic developmental kinetics and feto-maternal recognition in numerous species (Hammer et al. 2001).

Sexing pre-implantation embryos in wildlife assisted reproductive technology (ART) holds great potential as a conservation tool (Bora 2022). While there is a lack of specific references on endangered species, existing techniques utilized in breeding programs for sexing bovine and ovine embryos can be adapted and applied to wildlife species within the same families. By modifying these techniques, it becomes possible to manipulate the sex ratios of progeny and thereby contribute to the preservation efforts of endangered wildlife species (Andrabi and Maxwell 2007).
Various wildlife species have undergone trials regarding *in vitro* fertilization (IVF) but with varying levels of success (Herrick 2019). The prominent studies on reproductive assistance involving IVF are in tigers (Donoghue et al., 1990), cheetahs (Donoghue et al., 1992), pumas (Miller et al., 1990), Indian desert cats (Pope et al., 1993), Armenian red sheep (Coonrod et al., 1994), llamas (Del Campo et al., 1994), gaurs (Johnston et al., 1994), African elephants (Kidson et al., 1995), zebras (Meintjes et al., 1997), marmosets (Gilchrist et al., 1997), gorillas (Pope et al., 1997), minke whales (Fukui et al., 1997), bongos (Pope et al., 1998a), addaxes (Hall-Woods et al., 1999), sika deer (Comizzoli et al., 2001) African wild cats (Pope et al., 2000), jaguars (Morato et al., 2000), ocelots, tigrinas, blesboks, African buffaloes, springboks, black wildebeests (Herrick et al., 2004) and European mouflons (Ptak et al., 2002; Berlinguer et al., 2005) (Andrabi and Maxwell 2007).

Sperm cryopreservation and artificial insemination

The role of enclosed breeding is crucial in the species conservation that faces challenges in surviving in the wild. It serves as a valuable method for maintaining breeding stocks, which can then be utilized for reintroducing individuals into dwindling or extinct populations, aiming to repopulate and restore their numbers. By safeguarding these vulnerable species in captive environments, we can enhance their chances of long-term survival and contribute to the overall conservation efforts (Prieto et al. 2014).
The method of semen collection in avian species was initially introduced by Burrows and Quinn in 1935, employing domestic chickens as the model (Mohan et al. 2018). This ground-breaking technique has since paved the way for the establishment of semen collection and artificial insemination as widely practiced procedures. These procedures have found application not only in the poultry industry but also within captive breeding programs for both commonly found and endangered non-domestic species worldwide (Barna et al. 2020). As a result, these techniques have played a vital role in the conservation efforts aimed at preserving and increasing the populations of diverse wildlife species (Samour 2004). Sperm cryopreservation involves the preservation of spermatozoa by cooling them to extremely low sub-zero temperatures, typically reaching as low as the boiling temperature of liquid nitrogen at -196 °C (Murray and Gibson 2022). This process allows for long-term storage of sperm samples. To maintain acceptable viability of the preserved spermatozoa, it

is typically necessary to dilute them in cryoprotective agents (Tamburrino et al. 2023). These agents help protect the sperm cells during the freezing and thawing processes, ensuring their survival and functionality upon future use. By employing cryopreservation techniques, the viability and availability of sperm samples can be extended, enabling various applications such as artificial insemination, assisted reproductive technologies, and conservation efforts for both domestic and non-domestic species (Prieto et al. 2014). Interactions during thawing can be species-dependent, with cryoprotectants categorized as either membrane permeable (e.g., glycerol, ethylene glycol, DMSO) or impermeable (e.g., sucrose, trehalose, raffinose, PVP) (Prieto et al. 2014).

Sperm cryopreservation employs three main processes: very slow freezing, slow freezing, and vitrification (Riva et al. 2018). Each method utilizes specific cooling rates and protocols to minimize potential damage to the sperm cells during freezing (Prieto et al. 2014)

Spermatozoa preservation and their application in assisted reproduction procedures are valuable tools for breeding programs focused on rare and endangered species. To ensure success, it is crucial to adapt protocols to the specific conditions of each species. By tailoring techniques and procedures accordingly, we can optimize the effectiveness of assisted reproduction methods in conservation and breeding efforts for these vulnerable species (Fickel et al. 2007). Artificial insemination (AI) technology has evolved into a practical and widely used technique in commercial dairy cattle programs, both in developed and developing countries. Its application has significantly contributed to the improvement of breeding practices and genetic advancements in the dairy industry. Looking back in history, the first successful artificial insemination was conducted by Spallanzani in 1784, using a bitch as the recipient (Verma et al. 2012). The pioneering efforts in the field of artificial insemination (AI) can be traced back to Russia in 1899, initiated by Ivanow (Ivanoff, 1922). Ivanoff's groundbreaking work involved the study of AI in various domestic farm animals, including dogs, foxes, rabbits, and poultry (Verma et al. 2012).

AI plays a significant role in conservation by addressing the issue of genetic degradation resulting from the group fragmentation in free-living species (Penfold and Wyffels 2019). Through AI, genetic diversity can be preserved and enhanced by introducing genetic material from different individuals into fragmented populations (Andrabi and Maxwell 2007). While AI technology holds immense potential in conservation programs, it also faces notable limitations when applied to wildlife species (Holt and Comizzoli 2022). Although semen collection from most non-domestic mammals is generally achievable through techniques such as artificial vagina, vaginal condoms, digital masturbation of the penile bulb, or electroejaculation under anesthesia, these processes can be challenging for certain species. Rhinoceros, non-domestic equids, specific great apes, canids, and marsupials, for instance, pose difficulties in semen collection (Pukazhenthi and Wildt 2003)

AI technology has made significant advancements in wildlife, resulting in successful live births across various species. Notable achievements include the blackbuck (Holt et al. 1988), African lion (Bowen et al. 1982), Persian leopard (Dresser et al. 1982), black-footed ferret (Howard et al. 1991), ocelot, Eld's deer (Monfort et al. 1993), cheetah (Wildt et al. 1997), marmoset (Morrel 1997), Poitou donkey (Trimeche et al. 1998), Asian and African elephants(Olson and Wiese 2000; Brown et al. 2004), and giant panda (Masui et al. 1989). These successful outcomes demonstrate the effectiveness of AI in facilitating reproduction and species conservation efforts across a diverse range of wildlife species.

Oocyte cryopreservation and *in vitro* maturation

Oocyte cryopreservation is an effective approach for conserving the genetic potential of individual females (Argyle et al. 2016). However, recent outcomes have shown poor results in terms of viability, capability to fertilize, development of embryo and rate of conception. Additional studies are required to improve the success and effectiveness of oocyte cryopreservation (Tharasanit and Thuwanut 2021).

The continuous availability of viable and developmentally competent oocytes is crucial for the advancements achieved in recent years in *in vitro* embryo production (IVEP). Mammalian oocytes have a relatively short fertile lifespan, which highlights the significance of storing unfertilized oocytes. By storing unfertilized oocytes, a readily available source can be generated, enabling experiments to be conducted at convenient times. This aspect holds practical importance, particularly in establishing a gamete bank, which can be utilized to derive specific genetic combinations. The storage of unfertilized oocytes provides flexibility and opportunities for research, breeding programs, and conservation efforts, contributing to the overall progress in reproductive technologies and genetic management of animal populations (Verma et al. 2012). Cryopreservation techniques for oocytes, sperm, and embryos are typically categorized into two main methods: controlled-rate slow freezing and "ice-free" vitrification (Tharasanit and Thuwanut 2021).

Oocytes have low membrane permeability to both water and cryoprotectants (Leibo 1980; Edashige 201;). Despite advancements in freezing procedures that have led to improved oocyte quality, certain structures within the oocyte, such as the plasma membrane (Ashwood-Smith et al. 1988) and cytoskeleton (Saunders and Parks 1999; Shaw et al. 2000), are highly vulnerable to cryoinjury. These sensitive structures are often prone to cellular disruption and cell death during the freezing process.

Although there is significant interest in utilizing oocyte cryopreservation as a means of preserving genetic material from valuable animals, this technique is not yet widely established (Tharasanit and Thuwanut 2021). Challenges such as low survival rates, limited information on the post-thaw status of crucial biological attributes of oocytes, and inadequate developmental rates need to be addressed for further advancement. Continued efforts are required to refine and enhance oocyte cryopreservation techniques to make them more reliable and effective in achieving desired outcomes (Ledda et al. 2006).

Table 1 This table provides an overview of the evolution of assisted reproductive technologies in conservation biology from the 1970s to the 2020s.

	Period/ Decade	**Key developments**	**Significance**	**References**
1.	1970s	• First successful IVF in humans • Introduction of AI in livestock	• Pioneering technology • Initial adaptation to non-human species	(Biggers 2012; Lonergan 2018)
2.	1980s	• Development of embryo transfer • Cryopreservation of embryos and gametes	• Expansion of reproductive options • Long-term storage capabilities	(Mandelbaum 2000; Biggers 2012))
3.	1990s	• Advances in IVF techniques • Cloning of dolly sheep • First successful AI in endangered species	• Improved success rates • Milestone in animal cloning • Conservation application of AI	(Mastromonaco and Songsasen 2020)
4.	2000s	• Introduction of advanced genetic technologies (e.g., CRISPR) • Development of ICSI for ART • Increased focus on cryopreservation	• Enhanced precision in ART • Addressing male infertility • Preservation of genetic diversity	(O'neill et al. 2018; Sarma et al. 2024)
5.	2010s	• Expansion of ART to a broader range of species • Growth of collaboration efforts between zoos, conservationists, and biotech companies	• Application across diverse species • Improved conservation outcomes • Sharing of knowledge and resources	(Topaz 2016)
6.	2020s	• Advances in non-invasive ART • Integration of AI and machine learning in ART	• Reduced stress on animals • Data-driven decision-making in ART • Enhanced success and efficiency	(Raef and Ferdousi 2019; Fontana et al. 2024)

Cloning and genetic engineering

Cloning is a powerful technique with the potential to multiply elite animals and reduce genetic diversity in experimental animal models (Das et al. 2022). By producing genetically identical copies of a selected individual, cloning allows for the preservation and replication of desirable traits (Clarke and Merlin 2016). This technology offers opportunities for enhancing breeding programs, studying specific genetic traits, and advancing scientific research. However, it is important to carefully consider ethical, welfare, and genetic diversity concerns when contemplating the application of cloning in animal multiplication and experimental settings (Verma et al. 2012).

Recently, somatic cell nuclear transfer (SCNT) has emerged as a potential tool in conservation efforts (Iqbal et al. 2021). SCNT involves the transfer of the nucleus from a somatic cell of an endangered or rare species into an enucleated egg, resulting in the creation of a cloned embryo (Lanza et al. 2000; Latham 2004). SCNT may aid in the conservation and propagation of threatened species with poor captive reproduction until habitats are restored. It could also potentially resurrect extinct species from preserved tissue samples (Tong et al.

2002).

Application of assisted reproductive biotechnology

Assisted reproductive technologies (ARTs) for wildlife are suggested to address challenges in managing small, isolated animal populations with inconsistent reproductive outcomes (Pollastri 2023). It's important to note that ARTs are not meant to replace natural breeding but to complement it. Enhancing husbandry practices and promoting natural breeding remain fundamental in managing populations (Greggor et al. 2018). ARTs offer tools to support population management when natural breeding falls short (Clulow and Clulow 2016).

ARTs can help in situations where animals might not breed naturally due to behavioral differences, reducing the need to move animals between zoos for mating (Swanson 2023). The most significant potential of ARTs lies in overcoming space limitations and the availability of founding individuals (Herrick 2019). With cryopreservation of gametes and embryos, many individuals can be represented by samples stored in tanks, allowing for larger effective populations than zoos can accommodate (Comizzoli 2018). Additionally, wild gametes can be collected, preserved, and used for breeding in zoos, introducing new genetics without capturing animals from the wild. Periodically adding new founding individuals also helps maintain genetic diversity (Ballou et al. 2023).
While ARTs offer huge benefits to zoo breeding programs, efficient protocols are lacking for most endangered species and need to be developed individually for each species (Lueders and Allen 2020). In contrast, ARTs are widely used and well-established in laboratory rodents, livestock, and humans (Park and Sasaki 2020). Their routine use might make their complexity and the research behind them seem overlooked. Even basic techniques like artificial insemination require a deep understanding of female reproductive anatomy and physiology. Monitoring the estrous cycle accurately is crucial for detecting or predicting ovulation (Stevenson and Britt 2017). Methods to control ovulation timing without harming oocytes or the maternal environment are also needed. Understanding male anatomy and physiology is essential for collecting high-quality sperm at the right time (Ricardo 2018).

For *in vitro* fertilization (IVF), stimulating multiple follicles to produce more oocytes is necessary (Shrestha et al. 2015). These oocytes and sperm need to be cultured under conditions that support various stages of fertilization and embryo development until they're ready for embryo transfer, all while minimizing harm to the embryo.

Conclusions

Assisted reproductive biotechnology is of paramount importance in conservation biology. It proposes encouraging pathways to conserve the threatened species and ensure the protection of biodiversity. The massive challenges like low genetic variety and reproductive problems can be dealt with and overcome by the employment of advanced approaches. Assisted reproductive technologies can enhance conception rates, reduce the risk of genetic abnormalities, and help in the propagation of threatened species. Still, the ethical concerns and potential risks associated with their use must be kept under consideration. With continued studies, cooperation, and public support, we can take advantage of these technologies and strategies to protect our planet's intricate biodiversity.

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