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SYSTEMATIC REVIEW ON THE APPLICATION OF CELLULAR CULTURE TECHNOLOGY APPROACH IN CONSERVATION OF ENDANGERED SPECIES

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Abstract

The extinction of wildlife species is concerning. This issue has posed a threat to the whole biodiversity system significantly. Nevertheless, the extinction of wildlife species can be controlled and stabilized through a conservation program of *in situ* and *ex situ* approach respectively but in need of additional programs to save the species. This situation emphasizes the importance of *ex situ* conservation strategies, particularly cellular technology, in supplementing *in situ* efforts, which are becoming increasingly challenging. Cellular technology has an important role in conserving genetic diversity through optimized protocols such as assisted reproductive technology (ART). This paper conducts a systematic review and critical analysis of the current understanding of cellular technology in conservation. The study conducted an electronic search in three databases such as PubMed, ScienceDirect, and Scopus for articles published between 2010 and 2021. The search used keywords such as "cellular technology," "assisted reproductive technology," "cryopreservation," "conservation," "rescue," "save," and "endangered species." The findings suggest that assisted reproductive technologies are identified as cellular technologies for ex situ conservation efforts. However, the effectiveness of these technologies depends on various parameters specific to each endangered species. These parameters include genetic diversity, estrous cycle length, timing and method of semen collection, and the sperm quality and quantity. This review explores the application of cellular technology for conservation of endangered species and their future impact in conservation programs. More exploration is needed to fully harness the potential of cellular technologies in saving endangered species.

INTRODUCTION

The extinction of certain wildlife species is a natural and unavoidable evolutionary process. But today, human activities have prevailed, contributing to the declining population of wildlife. In 2020, the International Union for Conservation of Nature (IUCN) estimated that 40% amphibians, 26% mammals, and 14% birds are facing extinction [1]. The extinction of this species will affect the ecosystem, disturbing the food chain, especially if the species that extinct has major role in the ecosystem [2]. The length of food chain will be reduced if apex consumers population size is decreasing, and it will affect the intensity of herbivore and composition of plants. However, this effect will not be noticed until the species is reduced significantly or after they are completely lost from the ecosystem [2].

According to Emslie (2020), Northern White Rhino (*Ceratotherium simum ssp. Cottoni)* is listed as critically endangered because the population of this species has declined and at the brink of extinction as there are only two left of both which are female. It is believed that in 1960, there were 2,360 individuals Northern White Rhino, however, the number of Northern White Rhino decreased rapidly from 30 April 2003 due to excessive poaching. Other than the Northern White Rhino, IUCN (2020) also indicated that more than 35, 500 species including all species such as animals, plants and fungi are threatened with extinction. The most famous of endangered species across the world specifically animals are tiger (*Panthera tigris),* whooping crane (*Grus americana),* Asian elephant *(Elephas maximus)* and western gorilla (*Gorilla gorilla). Panthera tigris* is listed as endangered and it is estimated that approximately 3, 159 tigers are left in the world. The major factors that lead to the decline of this tiger population is due to poaching for illegal trade of body parts including skins, bones, meat and for medicinal use. Other than that, agriculture and silviculture, commercial logging and human encroachment also contribute to diminishing the availability of habitat for this species [3].

The aim of endangered animal conservation is to keep populations at large and diverse enough to be sustainable [4]. Human activity changes the environment too quickly for organisms to evolve in response, leading to extinction [5]. Restoring habitats will not stop the decline in biodiversity because many species are now fragmented, resulting in unviable populations with low genetic diversity [6]. Thus, many species are facing the possibility of becoming extinct due to human action as well as other several factors.

To maintain biodiversity, every species must be conserved, because by eliminating any species can cause an entire ecosystem to malfunction [7]. To rescue and conserve endangered species, researchers have developed many conservation approaches such as establishing protected areas, captive breeding and reintroduction, conservation legislation and increasing public awareness [8]. There are two primary approaches to conserve endangered wildlife species: *in situ* conservation and *ex situ* conservation. *In situ* conservation is the protection and management of species in their natural habitats, ensuring the survival of ecosystems and natural processes. This approach entails preserving germplasm and habitats on-site, allowing species to continue their natural cycles and interactions. *Ex situ* conservation, on the other hand, is concerned with the preservation of biological diversity components outside their natural habitats, which is frequently accomplished through captive breeding, seed banking, and cryopreservation. This method allows for the preservation of genetic diversity and species survival in a controlled environment, off-site from their natural habitat. The integration of *in situ* and *ex situ* conservation strategies is critical for overall species conservation. *Ex situ* conservation can sometimes supplement *in situ* efforts by serving as a genetic diversity reservoir and a source for reintroduction programs. One of the elements in *ex situ* conservation is the application of cellular culture technology in preserving the genetic of certain species. In wildlife conservation, cellular culture technologies play an important role in the survival of endangered species. These technologies include a variety of methods such as tissue culture, cryopreservation (biobanking system), and assisted reproductive technology (ART), all of which contribute to the preservation of genetic diversity and the survival of endangered wildlife species. Tissue culture techniques, such as the formation of cell lines and the growth of primary cells, are critical for preserving genetic material from endangered species. These techniques enable *in vitro* cell propagation and conservation, making them a valuable resource for genetic studies and potential reintroduction programs [9]. Furthermore, cryopreservation of gametes and embryos increases the fertility potential of endangered species by preserving valuable genetic material from animals that do not reproduce naturally or died prematurely [10]. This method is an important tool for preserving genetic diversity and preventing the loss of valuable genetic resources.

Assisted reproductive technology, such as somatic cell nuclear transfer (SCNT) and *in vitro* fertilization (IVF) enable the propagation and conservation of endangered species. These technologies allow offspring production from preserved genetic material, which helps to conserve species with limited population sizes and genetic diversity [11]. The use of cellular culture technologies in wildlife conservation goes beyond genetic preservation. These techniques also help with reproduction, captive breeding, and reintroduction efforts for endangered species. Captive breeding programs, which frequently rely on cellular culture technologies, have been instrumental in preventing species extinctions and providing a reservoir of genetic diversity for potential reintroduction into the wild [12].

The risks associated with cell culture technology in wildlife conservation are multifaceted and must be carefully evaluated. The challenges associated with the accessibility and cost of opportunistic technologies in wildlife management efforts indicate that the adoption of cell culture technology may be limited by practical and financial constraints [13]. Nevertheless, the inaccessibility of the terrain, the high costs associated with monitoring and enforcing conservation actions, and the difficulty of using available surveillance technology may impede the effective application of cell culture technology in wildlife conservation [14]. But with so many species being lost today, cellular technologies are essential tools in wildlife conservation, helping to preserve genetic diversity, captive breeding, and long-term management of endangered species. These technologies, combined with the incorporation of cultural and community perspectives, are critical for the comprehensive and effective conservation of wildlife. This review will discuss the application of cellular culture technologies in conservation of endangered species to resurrect biodiversity.

MATERIALS AND METHODS

Formulation of the Review Question

The review question was constructed based on the PICO formulation that is the population of the studies (P), intervention or exposure (I), comparison of intervention or exposure (C), and outcome of the interest (O) [15]. Thus, the formulated question for this review was "What are the cellular technologies that are used in conservation of endangered species?"

Search Strategy

An electronic search was conducted in three databases, namely PubMed, ScienceDirect and Scopus for articles published between 2010 and 2021. The search aimed to identify all cellular technologies used in conservation of endangered species. The following keywords were used in the search: "cellular technology" OR "assisted reproductive technology" OR "cryopreservation" AND "conservation" OR "rescue" OR "save" AND "endangered species". Identical search strategies were applied in all databases.

Inclusion and Exclusion Criteria

The inclusion criteria of this review were based on the PICO formulation, whereby P: endangered species population (animal), I: cellular technologies, C: worldwide and, O: conservation of endangered species. The types of studies included in this review are in English. The exclusion criteria were studies that did not focus on the PICO formulation, studies that focused on endangered species plant, studies that were in other language besides English and grey studies, case reports, letters, conference abstracts and review papers.

Data Extraction

The three stages of article screening (title, abstract and full text) were carried out independently by the author. The characteristics of the included studies (year and author, country of study, type of endangered species used, cellular technologies used and outcome of the study) were analyzed and summarized.

Assessment of Risk of Bias in Included Study

The methodological quality of the included studies was assessed using the Joanna Briggs Institute (JBI) Critical Appraisal Tools. This assessment determined the extent to which a study has addressed the possibility of bias in its design, conduct, and analysis [15]. The details of risk of bias assessment approach can be referred via the link below: https://jbi.global/sites/default/files/202110/Checklist_for_Systematic_Reviews_and_Research_Syntheses.docx.

RESULTS AND DISCUSSION

Studies Selection

Initially, a total of 125 articles matching the search terms were identified in the database search. The studies were then checked for duplicated studies. After removing the duplicates, 105 studies remained potentially eligible for inclusion. The study titles and abstracts of the 105 studies were screened to identify the relevant studies for inclusion in the review. Following that, only twenty studies were qualified for full-text screening. Out of the twenty studies, only 14 studies were eligible to be included in the review based on the inclusion criteria.

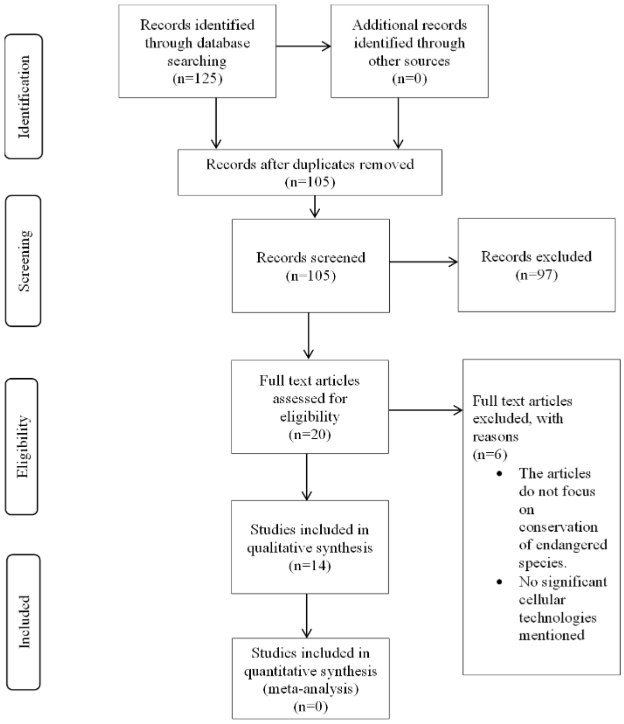

Figure 1. The flow diagram of the protocol based on the Preferred Reporting Items for Systematic Review and Meta-analysis (PRISMA) [23].

Cell Lines Derivation

Cell tissue culture is critical in wildlife conservation, particularly the preservation of endangered species. Somatic cell banks have been highlighted as a valuable approach for preserving genetic material from endangered wild felids from various continents. [16]. Furthermore, the cloning of endangered species using interspecies nuclear transfer has been investigated as a potential method for conserving rare or endangered vertebrate species, despite challenges in maintaining biodiversity through habitat and wildlife conservation [17]. The use of emerging technologies, such as cloning and stem cell-based technologies, has sparked debate about their practicality and applicability in the study and conservation of endangered species [16][17].

In addition to direct cell tissue culture application, the integration of cultural and community perspectives is critical for long-term wildlife conservation. Conciliating culture and wildlife conservation is critical for long-term community development projects and stakeholder well-being, emphasizing the importance of positive connotations to support human-wildlife coexistence [18]. Moreover, understanding local people's attitudes towards wildlife conservation can provide valuable insights into their behavior and willingness to coexist with wildlife, which is essential for developing effective conservation strategies [19]. These references emphasize the importance of cultural perspectives and community involvement in wildlife conservation efforts. On top of that, the practical application of disease risk analysis for reintroducing wildlife species has been emphasized as an effective tool for meeting wildlife management and conservation objectives, demonstrating the importance of risk assessment in conservation efforts [20].

Assisted Reproductive Technologies (ARTs)

Assisted reproductive technology (ART) includes a variety of techniques for manipulating reproductive processes, such as *in vitro* fertilisation, embryo transfer, and gamete preservation. In wildlife conservation programmes, ART is used to help preserve endangered species, maintain genetic diversity, and population sizes. Integrating ART into conservation efforts requires a multidisciplinary approach that combines advances in species biology, population research, gene banking, and biodiversity management to develop effective wildlife conservation strategies. The most common cellular technology that is used for conservation of endangered species is assisted reproductive technologies (ARTs) [21]. However, an efficient protocol for ARTs needs to be optimized regarding species, and some of available protocols were not applicable for many endangered species. According to Hildebrandt *et al*. (2018), assisted reproductive technologies techniques involve taking or collecting oocytes and spermatozoa properly and safely; oocyte in vitro maturation and fertilisation are achieved; the blastocyst stage can develop by stimulating the resulting zygote; and then the blastocyst is transferred carefully and efficiently into the uterus of a synchronous surrogate mother. In addition, the embryos can also be kept through cryopreservation to be used later, or may be processed as sources of stem cells.

Artificial Insemination (AI)

Artificial insemination (AI) is a reproductive technique used to help domestic animals and wildlife breed and reproduce. It entails the collection of sperms from a male animal, which is then processed, stored, and artificially introduced into a female animal's reproductive tract to achieve conception. AI is widely used in animal husbandry and conservation programmes for a variety of reasons, including genetic enhancement, disease control, and population management. Artificial insemination (AI) is a basic ART protocol that allows for the re-infusion and dissemination of genes even after the sperm donor has died when combined with semen cryopreservation. Using AI will reduce the likelihood of reproduction failing due to sexual incompatibility. However, the use of AI for endangered species is still limited due to the low survival rates of cryopreserved sperm, as freezing and thawing techniques have been shown to cause sperm quality or function impairments such as injury to the plasma membrane, reduced motility, decreased mitochondrial function, and low sperm survival in certain species [22]. Furthermore, the use of AI is dependent on knowledge of the oestrous cycle as well as research into semen collection and processing.

Cryopreservation

Cryopreservation is the process of cooling and storing cells and tissue at very low temperatures, so that the cells can be maintained for a very long period. Cryopreservation can be considered an essential procedure in assisted reproductive technologies, and is usually used further for artificial insemination and in vitro fertilisation [22, 23]. However. Cryopreservation processes may cause structural injuries or impairment to the membrane and to the genetic material of spermatozoa in several species [22, 23]. Other than that, the development of sperm cryopreservation protocols for endangered species is also difficult due to the highly ejaculate quality within individuals, for instance, like Asian elephants (Elephas maximus). This is because seminal plasma may influence the sperm physiology and function. Such condition will result in a decrease in artificial insemination's effectiveness to save this endangered species [23].

***In Vitro* Fertilization**

For *in vitro* fertilization (IVF), the growth of multiple follicles needs to be stimulated to increase the quantity of available oocytes. Then, the oocytes and spermatozoa should be cultured in a suitable environment that is suitable for sperm capacitation, fertilization, oocyte activation, cell division and embryo development. This condition needs to be preserved until the suitable time for embryo transfer with low impact on the survival of embryo. If there is any lack of these condition, the rate of success for IVF may be low [23]. In addition, the extra embryos can be kept to be used later either for transfer or processed as sources of stem cell by cryopreservation [21].

Semen Collection and Characterization

Semen collection requires a suitable and reliable method of gathering sperms to improve sperm quality. Using the most appropriate method to collect semen can contribute to the development of a better protocol for semen processing and cryopreservation, as well as increasing the success rate of endangered species conservation. Natural ejaculation, rectal massage, and electroejaculation, all of which require anaesthesia, are some of the methods available for semen collection [21, 24]. However, depending on the species and method of semen collection, the quality, quantity, function, and survival of sperm may vary [24].

Oestrous Cycle Characterization

To implement ARTs, it is necessary to understand basic reproductive characteristics of females, such as the length of the oestrous cycle and the ovulation period. Oocyte sample collection is usually triggered by hormones, and the ovulation period is predicted using ultrasonographic examination.

According to the literature review, the use of cellular technologies, such as assisted reproductive technologies, is primarily focused on clinical applications and research to improve the success rate of endangered species conservation. Furthermore, the research aimed to investigate appropriate elements and parameters of the components involved in the studies to develop a good protocol for rescuing endangered species [24]. The parameters of requirements for saving endangered species may be difficult to establish because each species has a unique set of genes, oestrous cycle length, timing and method of semen collection, and sperm quality and quantity [21, 23, 24]. Ultrasonography is commonly used to assess the length of oestrous cycle.

The assisted reproductive technologies, which include artificial insemination, *in vitro* fertilisation, and cryopreservation, are commonly used in combination [22]. Many endangered species were used in this research to discover ways to prevent them from becoming extinct, including the southern white rhinoceros, Abyssinian donkey, Oncilla (*Leopardus tigrinus*), and Asian elephants (*Elephas maximus*). These animals have significantly different genetic material from one another, so different methods and requirements may be used when dealing with them in the conservation of endangered species.

Ethical Implications

The ethical implications of incorporating cellular technology into wildlife conservation programmes are multifaceted and must be carefully considered. While these technologies have the potential to protect genetic diversity and prevent the extinction of endangered species, they also raise ethical concerns about animal welfare, conservation priorities, and the impact on natural populations. Assisted reproductive technologies, such as *in vitro* fertilisation, embryo transfer, and gamete rescue, may require invasive procedures and manipulation of reproductive processes in wildlife species. Individual animals may experience stress and discomfort during gamete or embryo collection, as well as changes to their natural behaviour and reproductive physiology. Prioritizing individual animal welfare is critical, ensuring that assisted reproductive technologies cause as little harm and distress as possible.

When discussing the ethical implications of using assisted reproductive technology (ART) in wildlife conservation programmes, it is critical to address a few issues. The use of ART may involve invasive procedures, hormonal manipulation, and artificial reproductive interventions, all of which raise concerns about the welfare of individual animals. Ethical considerations include minimising the animals' stress, pain, and discomfort during these procedures. While ART can help with genetic management and the preservation of endangered species, ethical considerations include maintaining genetic diversity and reducing inbreeding risks. Careful consideration of genetic implications and long-term population health is required. The use of ART may raise concerns about maintaining species integrity and natural reproductive behaviours. Ethical considerations include ensuring that ART interventions do not disrupt the species' natural behaviours or reproductive processes. Furthermore, ethical decision-making in wildlife conservation programs using ART involves prioritizing conservation.

Challenges and Future Perspectives

The challenges and prospects of cellular technology in conservation cover a wide range of topics, including genetic diversity preservation, species survival, ethical considerations, and technological advancements. Despite the promising prospects for preventing species extinction, there are some important considerations when implementing wildlife biobanking. Disease transmission may occur primarily through sample transport, as sampling under sterile conditions is not always feasible in field studies of wildlife. To reduce contamination and disease transmission from frozen material, donor animals should be screened early, and appropriate biosecurity protocols are implemented. However, it is worth noting that there has been no direct evidence of disease transmission from transferred cryopreserved animal embryos over the last 25 years. Furthermore, most studies have focused solely on the initial effects of conservation on gamete physiology, without considering the future application of ART. Planning an expanded study to make the best use of the collected samples should be considered in the future.

Research could concentrate on determining the long-term impact of ART on genetic diversity within populations, including the potential loss of genetic variation and strategies to reduce genetic homogenization. Successful use of assisted reproductive technology (ART) in wildlife, particularly in the context of genetic diversity preservation can be exemplified by successful conservation breeding of black ferret. Howard, *et al.* "Recovery of gene diversity using long-term cryopreserved spermatozoa and artificial insemination in the endangered black-footed ferret" (Animal Conservation, 2015) demonstrates the potential of assisted reproduction, including the development of 'genome resource banks'.

CONCLUSION

Advancements in cellular technology, especially in assisted reproductive contexts have led to significant progress in wildlife conservation over the past few decades. However, these techniques must be applicable. Therefore, effective global cooperation and action are required. It is crucial to share knowledge, data, and samples, as well as establish international biobank networks. Standardizing protocols for all techniques require global collaboration. This is especially important for research groups and biobanks with limited access to biological material to develop ad hoc protocols. Future conservation strategies should include viable tissue cryo-banking to provide genetically diverse material to combat extinction [44]. ART has the potential to help preserve genetic diversity and protect endangered species. Long-term monitoring and research are required to evaluate the health, reproductive success, and genetic diversity of offspring conceived using ART. This is critical for understanding the effectiveness of ART in promoting species survival and genetic diversity preservation. In conclusion, the use of cellular technology in wildlife conservation has the potential to preserve genetic diversity while also helping endangered species to survive. More research and collaboration among stakeholders are required to address the challenges and maximize the potential of ART in wildlife conservation.

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CONFLICT OF INTEREST

The authors declare that there is no conflict of interests regarding the publication of this manuscript.

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