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Biobanking of Endangered Mammalian Gametes and Embryos: A Review

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Abstract

The wealth of fauna resources has a strategic role in realizing the sovereignty and independence of the nation, so it needs to be preserved. Mammals contribute various ecological functions essential to human welfare, including sustaining energy flows and productivity and producing a different kind of biodiversity that is currently approaching extinction yet still exists. Animal biobanking is mostly focused on spermatozoa, oocytes, and embryos in various faunas, which is very relevant and an essential idea for producing healthy and sustainable populations. The quality of reproduction of local fauna with substantial economic value has also been increasingly degraded in recent years, so an accelerated effort in the field of conservation is needed, including integrating the latest technology with conservation efforts. However, the development of biobanking technology for endangered mammals still needs to be improved in its studies and innovations. The authors examine this issue since there is still information that has to be enhanced on the use of reproductive biotechnology techniques that center on the biobanking of animal gametes and embryos, particularly those of endangered mammals. This article provides information on the biobanking of sperm, oocytes, and embryos in endangered mammals.

Keywords: *Biobanking, Endangered mammals, Sperm, Oocytes, Embryos*

1. Introduction

The world has a biodiversity that is threatened with extinction, both in terms of habitat and population. This population reduction can be caused by forest land, which is their original habitat and has been converted into plantations and industrial land. The company's conversion of land around the conservation area is also a significant threat to conservation because of the opening up of access roads for hunters. The wealth of fauna resources has a strategic role in realizing the nation's independence, so it needs to be preserved. Various ecosystem functions are impacted by biodiversity, just as mammalian communities are crucial to maintaining ecological integrity. Mammals offer a range of environmental services that are critical to human welfare, such as sustaining productivity and energy flows and fostering biodiversity [1]. The main threat to all endangered hazards in their lives is habitat destruction and degradation of biological resources, many of which are currently extinct, and many more are on the verge of extinction [2].

The idea that biobanks are essential to different species is not a novel concept. This concept merits immediate and thorough conservation since it is highly relevant to the majority of biodiversity that is susceptible to epizootics and abrupt changes in social and political structures. In addition, the biobanking program serves as an archive for biomaterials, particularly germplasm, which helps conservation breeding initiatives create robust and long-lasting populations. Most early animal biobanking efforts were directed at spermatozoa and embryos [3] and female genetic material, especially oocytes, which are large and complex and require specific cryopreservation [4]. The most priceless items in the wildlife biobank—such as DNA, tissues, blood products, and reproductive cells—are crucial to understanding and preserving biodiversity.

Along with the development of the current era, the diversity of Indonesia's fauna is starting to be threatened. Habitat destruction and population decline due to illegal hunting and trade are happening faster than the effort to protect the fauna. The habitat of some fauna in Indonesia has decreased, resulting in isolated fauna and a high probability of inbreeding in these populations, such as the Banteng (Bos javanicus; status: endangered). Recent years have also seen a decline in the quality of reproduction of the local fauna, which is crucial to the economy. As a result, conservation efforts must be accelerated, incorporating innovative technology into both in-situ and ex-situ conservation efforts through the care and breeding of animals away from their natural habitat. Research and innovation in biobanking technologies for threatened species of mammals must be enhanced. Programs to manage species, such as frozen germplasm, can lower the required space; for instance, a partial dependence on frozen semen artificial insemination can reduce the number of live animals required in captive facilities and zoos by up to 50% [5]. The ex-situ artificial insemination method manages large-scale biobanking, like giant pandas, employing thawed, fresh, and stored spermatozoa [6]. Once considered the most endangered species in North America, the black-footed civet has adapted to modern times by combining artificial insemination with natural mating [7], as well as embryonic biobanking [8].

The process of storing biological materials at low temperatures in a way that makes it possible to recover them whenever needed is known as cryopreservation. The capacity to intentionally create and reverse low-temperature states in a way that reduces or fixes transition-related damage is essential for successful storage and cell survival. Two primary cryopreservation methods can be used: vitrification, which solidifies material into a glassy state without forming ice, and gradual freezing, which converts a liquid phase to a solid crystalline phase [9]. Information regarding the application of reproductive biotechnology methods that focus on the biobanking of animal gametes and embryos, especially endangered mammals, must be made available.

The review aims to shed more light on the use of appropriate reproductive biotechnology techniques in the gamete or embryonic biobanking program for mammals, particularly those that are endangered.

Biobanking On Sperm

A popular assisted reproductive technique that allows gametes with sufficient quality, viability, and developmental potential to be stored for an extended period is cryopreservation. Gametes from humans and other domestic animals have been preserved using this method over the years [10] to facilitate standard assisted reproductive technology operations [11]. Through the application of artificial insemination techniques [12] and sperm from males with the desired genetic advantage [13], cryopreserved sperm might hasten the pace of gene repair. Additionally, gamete cryopreservation has been utilized to keep endangered species from going extinct or help endangered animals reproduce [14]. The quality and viability of cryopreserved sperm are decreased by the cooling and heating processes used in cryopreservation, which also cause alterations in oxidative metabolism and increased formation of reactive oxygen species (ROS) [15]. With sperm cryopreservation technology that retains high viability, assisted reproductive technology can be used to save germplasm and generate animals with the appropriate genetic advantage that efficiently produce meat and milk. To protect endangered species, we examine the developments in gamete and embryo biobanking in this research. The male gametes of the severely endangered Italian sheep breed were cryopreserved and freeze-dried to create a genetic bank [16].

Reproductive programs, including in vitro fertilization (IVF), artificial insemination (AI), and related technology such as intracytoplasmic sperm injection (ICSI), can be used to boost the reproductive rate of endangered species [17]. In mammalian species, cryopreservation protocols for sperm are well-established [18]. Conventional cryopreservation in liquid nitrogen lowers the temperature until it reaches -196 °C, which causes cellular dehydration. Cryoprotectants must be used throughout this process to avoid cell harm from osmotic pressure and membrane shrinking [19]. However, because of the high cost and scarcity of liquid nitrogen, particularly in arid regions and developing nations, these storage conditions are not always possible.

Increased laboratory equipment and procedures as a result of technological advancements in a variety of biotechnology sectors have increased the caliber of outcomes. Because automated freezing results in excellent sperm quality after thawing, it is preferable over traditional procedures that use liquid nitrogen vapor for cryopreservation [20]. However, employing conventional and automatic controlled-speed freezing produced the same results in multiple experiments [21]. Then, associated cryopreservation techniques for sperm include slow freezing, fast freezing, and ultra-quick freezing [22]. The ultra-fast cryopreservation procedure known as vitrification is strongly suggested to minimize the danger of

damage to sperm cryopreservation caused by the physical effects of temperature drops and intracellular water crystallization.

In contrast to conventional freezing methods, the vitrification approach uses very high concentrations of permeable cryoprotectants in an aqueous solution to preserve sperm. The media employed during the development phase of this technique has been changed as an alternative to gradual freezing, mainly by adding non-permeable cryoprotectants to lessen cell damage during cryopreservation [23]. Without cryoprotectants, sperm vitrification has been done, and the outcomes have been encouraging [24]. Human osmotic stress resistance is modulated by this technique [25]. However, it has been noted that a significant percentage of nonviable and immobile sperm are produced during the vitrification process in cattle [26]. Furthermore, sheep's sperm quality is significantly lower than fresh sperm's [28], leading to lower sperm motility, viability, and plasma membrane function [29]. In pigs, vitrification also significantly reduces sperm motility, viability, and acrosome integrity while not affect the integrity and condensation of its chromatin.

According to the study's findings, lyophilization is a workable technique for conserving spermatozoa from the Pagliarola, a domestic sheep species that is endangered. By removing water through the process of freeze-drying semen, specimens are preserved in a state known as anhydrousness, which raises hopes for the development of an efficient lyophilization technology for sperm storage [30]. Furthermore, after being stored at room temperature, both cryopreserved and lyophilized spermatozoa could be used in suitable amounts to use ICSI to trigger the development of blastocyst-stage embryos. The generation of blastocysts from sheep oocytes following ICSI using the freeze-dried spermatozoa approach has just been reported for the first time [31]. The goal of this study was to create reliable and scalable procedures that would yield blastocysts of a suitable caliber. Similar to the previous treatment, the ICSI procedure was utilized to evaluate the spermatozoa's capacity for fertilization due to their lack of vitality after being rehydrated [32]. When spermatozoa with broken membranes are used as an activation factor in sperm loss [33], artificial activation becomes a necessary step before using ICSI [34]. A sperm cryopreservation process should consider several variables, including the cell volume, sperm plasma membrane composition, and the freezing curve of various mammalian species. The availability of laboratory equipment and specific protocols for semen collection and seminal plasma removal before cryopreservation may account for discrepancies in published study outcomes [35].

Biobanking On Oocytes

Cryopreservation is a preservation process at freezing temperatures for a certain period [36]. The goal of oocyte cryopreservation technology is to preserve frozen oocytes in a way that keeps their quality for future use. Patients may choose to enhance their oocyte count acquisition through oocyte cryopreservation. Oocyte cryopreservation technology is often used to maintain genetic sustainability [37,38]. The successful application of oocyte cryopreservation technology to date is a significant achievement in cryobiology [39]. The application of cryopreservation technology has made rapid progress and is increasingly popular in the reproductive sector in supporting the success of assisted reproductive technology programs [40]. Oocyte vitrification, presently an alternative to gradual freezing, is a technique for oocyte cryopreservation that involves a quick compaction process with a limited volume and a high concentration of cryoprotectants [41, 42]. High cryoprotectant protection can stop ice crystals from forming during vitrification, which could harm the oocyte [43, 44].

The vitrification method is recommended as an alternative in cryopreservation programs because, in contrast to gradual freezing, it does not produce ice crystals that could harm or even kill cells during the freezing process [45]. Via the vitrification process, oocytes are rapidly frozen at -196°C while utilizing a high cryoprotectant concentration to prevent intracellular ice crystal formation that could harm the oocyte and its organelles [46]. Since vitrification produces fewer ice crystals that could damage the oocyte, it is more practical and efficient, takes less time, and only needs a small amount of liquid nitrogen to freeze; it is a more successful method of preserving oocytes than slow freezing [47]. After vitrification, the rates of oocyte survival, fertilization, and embryo division were higher than those of gradual freezing [48]. The rate of chromosomal and spindle configuration aberrations was lower in vitrification than in slow freezing [49]. Spindle thread recovery was more efficacious [50] and occurred faster in vitrification than in slow freezing [51].

In assisted reproduction, oocyte cryopreservation technology is frequently used with in vitro maturation technology [52]. The use of a mix of vitrification methods and in vitro oocyte maturation is frequently investigated in terms of the process, duration of storage, and impact on the capacity of fertilization and embryo development to yield the desired generation [53]. The application of vitrification technology combined with in vitro maturation is still being developed for optimal achievement rates [54].

Combining oocyte maturation and vitrification techniques can produce low fertilization and cleavage rates [55]. Vitrification of oocytes at the germinal vesicle stage followed by in vitro maturation is an alternative cryopreservation method in oocyte maintenance because the germinal vesicle stage is considered more efficient and tolerant of the effects of stress during vitrification compared to vitrification at the metaphase II stage [56]. However, other researchers have suggested that vitrification at the germinal vesicle stage not only impairs the ability of the oocyte to reach maturity but also induces ultrastructural changes in the meiotic spindle, thereby impairing its ability to align chromosome arrangements in the correct plane [57]. Other researchers added that a decrease in oocyte developmental competence after vitrification at the germinal vesicle stage might occur due to the mechanism of apoptosis initiation and reduction of cytoplasmic mRNA [58]. The findings from these investigations suggest variations in the average molecular alterations resulting from treatment of oocytes in vitro and in vitro maturation in combination. The combination of vitrification and in vitro maturation techniques can still reduce oocyte competence, as researchers reported that vitrification treatment on oocytes could cause changes in maturation rates and protein expression [59], changes in oocyte ultrastructure [60], changes in gene expression [61] and changes in deoxyribonucleic acid [62].

The decrease in oocyte maturation rate after vitrification is an increase in oocyte intracellular ROS associated with impaired mitochondrial function, as reported by other researchers [63]. The balance of ROS content is vital to maintaining oocyte growth, but excessive ROS production causes oxidative stress that affects oocyte quality. Like the role of the source of ROS (H_2O_2), at lower conditions, it can induce the resumption of meiosis in germinal vesicle stage oocytes but inhibit the extrusion of polar bodies in mature oocytes [64]. Still, the increase in ROS is associated with cell cycle arrest processes and events. Apoptosis [65], DNA fragmentation [66], and chromosomal aberrations during meiosis [67] Changes in temperature during vitrification in the critical zone during cooling can impact microtubule irregularity [68], which plays a vital role in the separation, arrangement, and movement of chromosomes [69]. Vitrification causes changes in protein expression reaching mature oocytes caused by changes in temperature during vitrification and the type and duration of immersion of oocytes in vitrified solution [70]. The achievement of oocyte maturation levels that are not optimal after vitrification and maturation may also be influenced by using oocyte devices [71].

Oocyte vitrification technology combined with in vitro maturation shows the complexity of signal transduction changes that affect oocyte competence. Adding a combination of hormone concentrations in in vitro maturation media significantly increased the percentage of oocytes reaching maturity, normal single configuration, normal chromosome arrangement, cortical granule migration, and mitochondrial aggregation [72]. However, the addition of inappropriate hormone concentrations in vitro can still interfere with oocyte maturation, including disordered chromosomal arrangement and abnormal spindle organization, leading to abnormal embryonic development [73]. Optimizing the quality level of oocyte maturation after vitrification combined with in vitro maturation needs to be improved by adding supplements or relaxants, as reported by other researchers [74,75]. Adding various antioxidants to the media can also be a strategy for protecting oocytes from oxidative stress during in vitro maturation [76]. Appropriate and effective methods are needed during oocyte cryopreservation to eliminate the toxic effects of cryoprotectants, avoid ice crystal formation, and avoid osmotic stress. Pre-treatment (before vitrification) can be a strategy for increasing vitrified oocyte survival rate and developmental competence [77]. Pre-treatment (before in vitro maturation) can effectively reverse the detrimental effect on the spindle [78]. Furthermore, adding antifreeze protein as a supplement in vitrified media has a protective impact on maintaining spindle integrity to increase oocyte survival rate [79].

Biobanking on Embryos

Cryopreserved embryos can be stored for an extended period, depending on the method used. The nitrogen tube used for cryopreservation must be maintained through careful handling, internal temperature control, nitrogen level maintenance, and storage in an appropriate location. Regarding the potential impact of longer storage times on the viability of embryos, no information is available. It is commonly accepted that in cells kept in liquid nitrogen (-196 ^{o}C), cell metabolism and biochemical reactions are either halted or reduced to negligible levels.

Gene expression profiles were altered in embryos that underwent the cryopreservation procedure due to exposure to heat and mechanical, toxic, and osmotic stresses [80]. Regardless of the embryos' in vitro or in vivo origin, the global gene expression of fresh and cryopreserved embryos revealed distinct variations in freezing-induced gene expression [81]. Furthermore, cryopreservation techniques—slow freezing or vitrification—can modify the expression of embryonic genes in distinct ways [82, 83]. Increased expression of genes linked to inflammatory processes, including immune cell trafficking, cellular movement, cell-cell signaling, and cell death, has been observed in cryopreserved embryos

[84]. The cryopreservation method requires the selection of embryos based on quality, and typically, the embryo's morphology is the most reliable indicator of embryo quality [85]. A high-quality embryo should have a well-formed blastocoel and inner cell mass, be at the proper developmental stage, and show no degeneration symptoms. Shape, color, number and compactness of cells, size of perivitelline space, number of extruded and degenerated cells, and number and size of vesicles are standard parameters to assess embryo quality [86]. Other invasive factors, such as total cell count, apoptosis, differentiation of cell lineages, screening of embryonic biopsied cell genomes, or intelligence-based prediction of embryo quality, can also indicate embryo viability [87].

Embryos created by in vitro systems lack the same quality and competency as those produced physiologically. However, several traits linked to a lower rate are present in blastocysts generated in vitro, including vacuoles in trophoblast cells, a sparse population of microvilli, fewer cell-to-cell contacts, variations in gene expression, and modifications in lipid metabolism [88,89]. The improvement of embryo manufacturing techniques and cryopreservation is one of the primary obstacles to assisted reproduction, as it is necessary to obtain acceptable pregnancy and survival rates after embryo cryopreservation. Cryopreserved embryos currently make up around 33% of in vitro embryos, while over 50% of in vivo embryos are of this type [90].

As compared to in vivo sources, in vitro technological methods continue to generate embryos with several defects that lower their quality and survival. Different transcriptional profiles, sparse microvilli populations, a reduced cell count, an increase in lipid droplet content, an excess of energy substrate metabolism, and high heat sensitivity are all contributing factors to this low embryo competence [91]. Based on an embryo-focused approach, some parameters, including in vitro culture systems and circumstances, culture media composition, growth factors, proteins, hormones, cytokines, and antioxidant supplements, influence embryo quality and survival rates following cryopreservation [92]. The total number of cells and a sufficient percentage of the inner cell mass are necessary for embryo development. In some species, the trophectoderm appears to dictate developmentally appropriate measures to minimize early embryo loss and to boost implantation and live birth rates [93,94]. When compared to non-resistant embryos, bovine embryos with a more significant total number of cells had a higher rate of re-expansion and hatching following vitrification [95]. Two distinct cryopreservation methods' effects on two additional species revealed that vitrifying blastocysts could result in more excellent survival rates and reduced cell apoptosis in rat and mouse blastocysts [96].

The rise in lipid droplets observed in in vitro embryos is another characteristic that is frequently linked to worse cryopreservation yields. All tissues include lipids, an energy storage source, and structural and functional elements of cell membranes that keep them fluid. Although the exact process underlying the accumulation of increased lipid content in embryos is unknown, the supplementing of embryonic culture material is typically linked to this rise. Numerous other factors will affect embryo quality, but the in vitro culture methodology and the freezing process have the most effects on the embryonic lipid profile [97]. The processes behind survival following cryopreservation are intricate and still mostly unknown. It has been shown that the buildup of lipids in the cells of in vitro embryos, particularly lipid droplets made of triglycerides, makes them more susceptible to freezing [98,99]. The lipolytic agent forskolin was utilized for 48 hours in culture before vitrification to increase embryo viability after transfer, which was the cause of the increased cry tolerance and fruitful pregnancy in B. indicus embryos in vitro [100].

Many biological and management-related elements, including oocyte and embryo developmental competence, semen quality, nutrition, hormone concentration, reproductive procedures, and others, are involved in the formation of pregnancy [101]. Yes, the majority of miscarriages happen in the early stages of embryonic development, including factors that are particular to the male, the donor, and the embryo. But to display reproductive features, some additional factors, including seeds, nutrition, and other necessary components, must be considered [102]. A population can benefit from enhanced reproductive efficiency and genetic benefits through the widespread use of assisted reproductive technologies. One of the most important metrics used to assess bull fertility and reproductive efficacy in artificial insemination nowadays is the male fertilization rate [103] because the genetic improvement of livestock relies on the use of genetically better males [104]. Several studies suggest little benefit from increasing female fertility because selection and management of male reproduction are the main stages for increasing production [105,106]. In dairy cattle, it was shown that the preimplantation of embryos from males with a low conception rate of males showed a decreased capacity to produce pregnancy [107].

4. Conclusion

Challenges of biobank conservation and biobank research that focuses on conservation and study face various challenges such as facilities for sample cryopreservation, implementing procedures and employing personnel to process samples, and financial capacity to ensure long-term sustainability. Raising awareness of the significance of this biobank in terms of research utilization, contributions to animal conservation, and the current and potential socioeconomic consequences of existing collections is contingent upon the support of the public and end users. If long-term sustainability is to be attained, the biobank's value must also be raised. Additionally, end users must work with the biobank to help acquire funding to purchase samples.

It has been claimed that the combination of in vitro maturation with the vitrification method of cryopreservation technology offers numerous advantages for use in assisted reproductive technologies. Enhancing the quality of sperm, oocytes, and embryos requires optimizing the use of vitrification medium in conjunction with the efficacy of in vitro maturation techniques in the lab. Over the past few years, novel biotechnics has truly revolutionized animal reproduction. Among other advantages, these instruments can facilitate faster genetic acquisition, more effective reproduction, scheduling of the best times for reproduction, and managing animal hygiene. A few decades ago, in vitro embryo manufacturing achieved unfathomable traction, and evidence now available indicates that its application will only increase in the years to come. The success of cryopreservation and maintenance during pregnancy depends on several factors related to the quality of gametes and embryos and competence development.

Furthermore, improvements in cryopreservation techniques are essential to obtain more satisfactory and economically justified results so that all the benefits of these techniques can be fully enjoyed. Both gametes and embryos must be healthy and prepared at the cellular and molecular levels to survive all the challenges due to cryopreservation procedures. Management and standardization of laboratory routines are crucial to maintaining the quality level of cryopreservation results. Testing each new reagent replaced, checking the osmolarity and pH conditions of the culture media, and proper professional training can produce better results than many modified cryopreservation protocols.

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Conflict of Interest

The authors declare that they have no competing interests.

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