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Cold and Fertile: Cryopreservation as an Innovative Tool in Addressing Challenges in Teratozoospermia

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Abstract

Teratozoospermia is an infertility issue that affects a significant number of couples of reproductive age. One of the potential causes contributing to the global decline in seminal quality includes factors such as diet, alcohol and tobacco consumption, high levels of stress, and environmental influences. This underscores the need to preserve the fertility of these patients through cryopreservation techniques. In this review, we explore the latest methods for freezing seminal samples, highlighting their advancements and advantages in addressing the challenge of perpetuating animal species, particularly in the context of human infertility.

Keywords

Cryopreservation, Teratozoospermia, Infertility

1. Introduction

Cryopreservation has demonstrated significant potential in conserving endangered species and addressing infertility issues in humans. This technique offers the possibility of delaying motherhood and storing genetic material indefinitely. Recently, cryopreservation of ovarian follicles, embryos, oocytes, and sperm has been observed and proven to provide solutions to various challenges faced in both animal species conservation and reproductive health applications in general [1]. While preserving natural habitats remains the optimal strategy for biodiversity conservation, there are instances where this option is not feasible at the desired level. Therefore, it is important to recognize complementary strategies for biodiversity conservation, such as the development of genetic resource banks and the use of reproductive biotechnologies [2]. As a result, genetic material

conservation through freezing becomes a viable means of preserving current genetic variability, offering an attractive option for the future by maximizing the conservation of genetic resources, including female gametes [2].

2. Teratozoospermia: An Adverse Condition in Male Infertility

2.1. General Principles

Teratozoospermia, a condition affecting around 9% of couples worldwide, emerges as a prominent cause of male infertility, contributing to 30% of infertility cases in couples. This pathology is characterized by the presence of sperm with structural abnormalities and is often associated with defects in sperm DNA. The impact goes beyond altered morphology, as affected sperm exhibit elevated levels of DNA damage and issues with DNA compaction [3].

This condition has diverse causes, some of which include the presence of varicocele, harmful environmental factors, infections in the reproductive organs, and endocrine disruptions [4]. It has been observed that a proportion of teratozoospermia cases are linked to defects in spermatogenesis. Defective spermatogenesis contributes to the genetic heterogeneity of teratozoospermia, and although genes associated with teratozoospermia have been identified, the etiology still remains unknown in numerous instances [4] [5].

The consumption of substances such as marijuana has also been linked to alterations in spermatic patterns and parameters, including morphology, motility, and sperm viability. International literature supports an increase in the number of abnormal sperm forms, reaching teratozoospermia, as well as a reduction in motility, including severe cases of asthenozoospermia [6].

Teratozoospermia manifests in various forms, encompassing different types of abnormalities in sperm morphology. The presence of sperm with atypical morphologies can be classified according to specific criteria, such as the presence of multiple nuclear anomalies, lack of acrosomal segmentation, or deformities in the tail. Additionally, the degree of impairment can vary, from mild cases with a moderate percentage of morphologically normal sperm to more severe forms with a significant prevalence of malformations. These varied expressions of teratozoospermia underline the complexity of this condition and the need for an individualized approach in the evaluation and treatment of associated infertility. According to the Sixth Edition of the WHO Laboratory Manual for the Examination and Processing of Human Semen, it is established that the minimum reference value for alterations in human semen morphology is at least 4% of sperm with appropriate morphology [7].

Furthermore, sperm DNA damage can be classified into three main categories: direct DNA damage, nucleus-related genetic mutations resulting in decreased DNA compaction, and irregular chromatin structure [3]. These phenomena not only compromise the fertilization capacity of sperm but are also associated with adverse consequences in embryonic development and pregnancy

outcomes. Complications such as pregnancy loss, recurrent spontaneous abortions, and reduced live birth rates are among the observed issues.

It is crucial to note that, although some genetic mutations related to sperm DNA damage have been identified, further research and clinical trials are required to understand the underlying mechanisms fully and develop more effective approaches to address teratozoospermia.

2.2. Worldwide Disease Scenario

Infertility is a global challenge that affects a significant number of couples worldwide, with an estimated 72.4 million experiencing fertility issues. According to the WHO, between 60 and 80 million couples are currently dealing with infertility, figures that range from 8% to 12% of couples worldwide and vary based on geographic region [8]. Male infertility plays a significant role in this landscape. Detailed research on sperm alterations indicates that low concentration (oligozoospermia), poor motility (asthenozoospermia), and abnormal morphology (teratozoospermia) are the main factors affecting fertility [8] [9] [10], with a more pronounced impact in less industrialized regions where infectious diseases also contribute substantially.

In the specific case of Mexico (see **Figure 1**), studies suggest that teratozoospermia affects up to 92% of infertility patients, followed by oligozoospermia at 32% and asthenozoospermia at 22% [9]. It is essential to consider that there is a connection between teratozoospermia and DNA alterations, with an incidence that could reach 53%, adding complexity to the landscape of male infertility. In the United States, approximately 10% of couples are considered infertile after 12 months of unprotected sexual intercourse. Additionally, the WHO notes that the fertility rate in men under 30 has experienced a 15% decline globally [8], highlighting the diversity and breadth of this challenge that affects couples in different cultural and geographic contexts.

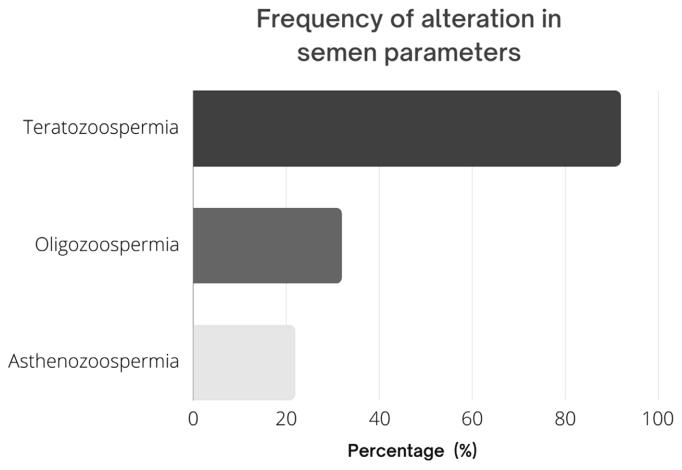

Figure 1. Frequency of alteration in semen parameters in Mexico. Teratozoospermia affects up to 92% of infertility patients, followed by oligozoospermia at 32% and asthenozoospermia at 22% [9].

3. Cryopreservation Techniques

Cryopreservation emerges as a crucial tool in the treatment of teratozoospermia due to the need to preserve cells from patients who, for various reasons, require fertility treatments and suffer from this condition. This technique stands out for its ability to preserve cells and tissues at extremely low temperatures, halting metabolic activity and allowing for long-term storage without degradation. In the context of teratozoospermia, cryopreservation becomes an invaluable ally in addressing the challenges of sperm quality [11].

The application of such low temperatures, ranging from −80˚C to −196˚C, forms the basis of this technique, with the potential risk of cell damage due to ice crystal formation. However, the strategic use of cryoprotectors minimizes these detrimental effects. These cryoprotectants have the function of preventing the formation of ice crystals and avoiding cellular dehydration during thawing, optimizing the viability of spermatozoa [12], and protecting the sperm from the toxic action of cellular metabolism products and temperature changes [13].

Moreover, they provide protection to the cell during the dehydration process, either by replacing the cell's water content in the case of permeable cryoprotectants or by stabilizing the membrane during the cryopreservation process in the case of non-permeable ones [14]. Biochemically, it is possible to distinguish three types of cryoprotectants: alcohols (methanol, ethanol, propanol, 1 - 2 propanediol, and glycerol), sugars (glucose, lactose, sucrose, saccharose), and dimethyl sulfoxide (DMSO). Cryoprotectants can also be classified into non-penetrating or high-molecular-weight agents and penetrating or low-molecular-weight agents, according to cellular permeability [11].

In the context of teratozoospermia, this technique offers tangible hope by allowing the preservation of semen samples with abnormal morphology, providing opportunities for assisted fertility treatments and the preservation of reproductive capacity. Therefore, it stands as a fundamental pillar in addressing reproductive challenges such as teratozoospermia.

Below, we discuss the main cryopreservation techniques that have shown better results in the context of sperm cryopreservation.

3.1. Vitrification

Vitrification is an extremely rapid cryopreservation method in which cells are immersed in a highly concentrated solution of cryoprotectants, solidifying during cooling without the formation of ice crystals. This technique is achieved by the direct immersion of cells in liquid nitrogen, representing a cooling rate of approximately 2500˚C /min and taking only a few seconds to be cryopreserved. During vitrification, the solution does not crystallize but vitrifies, meaning it abruptly increases its viscosity and transforms into an unstructured solid state similar to glass. Vitrification preserves both the molecular and ionic distribution of the liquid state. In the process, a clear-looking solid structure forms, which transforms back into a liquid state without the presence of a milky appearance

observed in crystallization or ice formation processes [11] [15].

3.2. Slow Freezing

Slow freezing is a process designed to prevent intracellular ice formation or minimize the damage it can cause. This process is based on partial dehydration of cells through exposure to solutions containing low-molecular-weight cryoprotectants such as ethylene glycol, glycerol, dimethyl sulfoxide, or propylene glycol. Adding cryoprotectants in a single step provides equal survival rates as adding them in multiple steps, while also accelerating and simplifying the process [16] [17].

The addition of the cryoprotectant produces transient changes in cell contraction and expansion as the cryoprotectant penetrates the cell, reflecting its osmotic response to a hyperosmotic environment [16] [17].

Cellular response to temperatures below 0˚C depends on the cooling rate to which the cells are subjected. If the cooling rates are very slow, a solution effect occurs, causing a decrease in cell volume and destruction of the plasma membrane. On the contrary, if the cooling rate is fast, intracellular ice crystals form, which can compromise cell viability during subsequent thawing [16] [17].

3.3. Vitrification in Pearls

In this process, spermatozoa are mixed with a concentrated solution of cryoprotectants, forming small pearls that are directly submerged in liquid nitrogen. Vitrification in pearls stands out for its speed, avoiding the formation of ice crystals and transforming the solution into a solid state similar to glass [18].

This technique offers several significant benefits. Firstly, its extreme speed minimizes the exposure time of spermatozoa to stressful conditions. Additionally, it achieves a high survival rate of spermatozoa after thawing, maintaining their viability and fertilizing capacity. By preventing the formation of ice crystals, the risk of cell damage is reduced, preserving the integrity of the spermatozoa.

Despite these benefits, it is important to note that vitrification in pearls requires specialized equipment and technical expertise. Cryopreservation of spermatozoa through vitrification in pearls represents an efficient option for preserving male fertility. However, a significant limitation of this technique is that it is an open system, so samples are in direct contact with liquid nitrogen, increasing the risk of cross-contamination between samples.

4. Discussion and Conclusions

Teratozoospermia is recognized as a widespread issue affecting couples worldwide, substantially contributing to male infertility and representing a significant percentage of infertility cases. This pathology, characterized by structural abnormalities in sperm, extends its impact beyond mere morphological anomalies, incorporating damages to the DNA structure. This intricate relationship involves

the entire spectrum of sperm morphology, demanding nuanced evaluation and a personalized treatment approach [8] [9] [10].

The global landscape of infertility positions teratozoospermia as a complex concern affecting millions of couples, with prevalence rates varying depending on geographical locations and social contexts [8] [9]. The stark reality that male infertility plays a fundamental role in this landscape requires a profound understanding of its etiological factors and a concerted effort to design specific interventions.

Hence, cryopreservation techniques are presented as pioneering solutions to the challenges posed by male infertility. The delineation of vitrification and slow freezing techniques provides a comprehensive understanding of the methodologies employed to preserve sperm viability for various patient needs. It is noteworthy that the innovative technique of vitrification in pearls emerges as a focal point, offering a representative freezing speed and efficiency in sperm preservation.

The advantages of vitrification in pearls, including minimal exposure time, higher survival rates post-thawing, and preservation of sperm integrity, position it as a formidable competitor in male fertility preservation. However, the recognition of its limitations, particularly its status as an open system susceptible to cross-contamination, requires further refinement and consideration in clinical applications [18].

Finally, it is important to note that the application of various sperm cryopreservation techniques has different economic implications. Slow freezing stands out for its initial accessibility, but may incur higher long-term costs due to continuous maintenance at low temperatures. On the other hand, vitrification, despite potentially higher initial costs due to increased cryoprotectant usage, offers greater sperm survival and requires less storage space. Vitrification in pearls, with its efficiency and speed, presents economic advantages by reducing costs associated with laboratory time, although specialized equipment incurs significant initial expenses. The choice between these techniques will depend on economic factors, preservation goals, and the laboratory infrastructure, carefully considering the detailed direct and indirect costs of each method.

In conclusion, the exploration of teratozoospermia and its intersection with advanced cryopreservation techniques provides a multifaceted understanding of challenges and solutions within the realm of male infertility. Teratozoospermia, with its intricate manifestations and implications for fertility, underscores the need for personalized diagnostic and therapeutic approaches. The global landscape of infertility, with millions of couples grappling with reproductive challenges, accentuates the urgency for innovative interventions.

Cryopreservation, particularly the cutting-edge technique of pearl vitrification, emerges as a promising avenue to address the adverse effects of teratozoospermia. However, the recognition of its susceptibility to cross-contamination emphasizes the imperative to refine this technique for broader clinical applicability.

Finally, it is essential to mention that the intricate interaction among genetic factors, sperm morphology, and cryopreservation requires ongoing research to fully unravel the underlying mechanisms. As the complexities of male infertility are delved into, the synergy between scientific research and technological advances becomes paramount.

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Conflicts of Interest

The authors declare no conflicts of interest regarding the publication of this paper.

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