Poonam Shodh Rachna(ISSN 2456-5563)
(A Multidisciplinary, Peer Reviewed and Refereed Research Journal)
Vol. 3, Issue.V, May 2024, PSR-2305041

Comparative Analysis Of Sperm Motility Post-Thawing In Cryopreserved Semen: A Review Of Method, Outcomes And Implications For Fertility Treatment

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Abstract

Introduction: *Semen cryopreservation is a basis of modern fertility treatments, offering invaluable benefits for assisted reproductive technologies (ART) such as in vitro fertilization (IVF) and intracytoplasmic sperm injection (ICSI). However, the preservation process can adversely affect sperm motility, a critical determinant of successful fertilization. This review critically examines and compares the effects of two prevalent cryopreservation method slow freezing and vitrification post-thaw sperm motility, utilizing data from sibling semen samples to ensure reliable comparative insights.* ***Background:*** *The analysis begins with an overview of the biological principles underpinning semen cryopreservation, highlighting the mechanisms through which cryo-damage occurs and affects sperm function.* ***Methodology****: It then delves into the technical aspects and procedural distinctions between slow freezing and vitrification, discussing the advantages and limitations of each method in preserving sperm motility and overall viability. Recent studies demonstrate that vitrification, though less commonly used, often results in higher post-thaw motility rates compared to slow freezing, potentially due to reduced ice crystal formation and faster cooling rates. In exploring the clinical implications, the review emphasizes the significance of post-thaw motility on ART outcomes.*

Advancement: *Enhanced motility can improve the success rates of procedures like IVF and ICSI, directly influencing fertility treatment efficacy. Furthermore, the review considers patient-specific factors, such as initial semen quality and age that may affect cryopreservation outcomes and suggests personalized approaches for optimal results. Future directions for research and practice are also discussed, including innovations in cryoprotectant formulations and thawing protocols aimed at minimizing cryo-damage. Advanced assessment tools for sperm motility are proposed to refine evaluation methods and improve clinical decision-making.* ***Conclusion:*** *In conclusion, this review underscores the necessity for continuous advancements in cryopreservation techniques to enhance post-thaw sperm motility, thereby improving the success rates of fertility treatments and offering better reproductive outcomes for patients.*

Key Words: *Semen Analysis, Sperm Survival, Sperm Motility, semen thawing*

Introduction:

The dropping birth rate is one of the most important societal challenges confronting affluent countries today, while it is widely overlooked that the number of infertile couples in these countries is increasing. While social (i.e., social progress for women and the resulting increase in the age at which women marry) and environmental (i.e., pollution and global warming) factors contribute to the rise in the number of patients with infertility, infertility in the male partner accounts for roughly half of all cases.(1)Reproductive disorders and infertility affect approximately 10-15% of couples worldwide. Successful reproduction requires precise regulation of complex processes such as gonad and reproductive organ development, sex determination, gametogenesis, neuroendocrine competency, and pregnancy capacity. Oogenesis is the process by which a mammalian egg becomes fertile. It involves complicated interactions between the oocyte and the somatic cells that surround it, as well as the interplay of several transcriptional regulatory

factors (2) Infertility in males may arise from various factors such as (factor name) is commonly assessed through a semen evaluation in andrology lab. The evaluation involves observing semen volume, concentration, sperm motility, epithelial cell is leukocytes, and sperm shape (morphology). An atypical semen analysis result does not give in depth information about male infertility (3).

Spermatozoa freezing, often known as cryopreservation, was first used as a fertility preservation technique in the 1960s (4). As a backup plan, freezing human semen is a crucial step in maintaining the fertility of cancer patients and those receiving infertility treatments. Due to geographical and medical obstacles, even some fertile couples may encounter problems becoming pregnant. Another crucial sign that sperm banking is necessary is a general decline in male reproductive health over time (5,6). The treatment for male factor infertility has fully evolved with the development of intracytoplasmic sperm injection (ICSI) and in vitro fertilization (IVF). Thus, cryopreservation via sperm freezing has emerged as a successful treatment option (7). .Cryopreservation is a procedure where cells, tissues or other substances vulnerable to chemical damage over time are preserved by cooling at -196^{0}C temperature. In 1957, the cryopreservation of tissues began with the freezing of fowl sperms (**8**).Cryopreservation was applied to humans in the mid-1950s (6). Cryopreservation is currently the most effective method for preserving sperm in males receiving gonadotoxic treatment, such as radiation or chemotherapy (9, 10), which can cause ejaculatory dysfunction or testicular failure. Moreover, sperm cryopreservation is available for certain non-cancerous illnesses that may impair reproductive processes, such as diabetes and autoimmune diseases (11). Lazaro Spallanzani first noticed reducing the spermatozoa temperatures in 1776 (12).He saw that whereas vs now significantly slowed down sperm motility, sperm resumed their previous level of motility upon reheating. Prior to 1949, numerous scientists documented that while sperm could withstand freezing, only a small percentage of them were able to fertilize the oocyte thereafter. When Polge et al. discovered a way to protect sperm damage after freezing in 1949, they set the stage for the cryopreservation of human sperm.In 1953, Dr. Jerome K. Sherman used improved techniques to successfully create the first human pregnancy with frozen spermatozoa (13). In contrast to the pre-freeze state, sperm motility, plasma membrane functioning, acrosome integrity, and viability usually decrease in the post-thaw stage (14). According to Nijs et al., following cryopreservation, the proportion of motile spermatozoa d ropped from 30% to 50% (15).The purpose of this study is to ascertain. It is commonly recognized that sperm shape and function are adversely affected by cryopreservation. Recovering the optimal amount of functionally intact spermatozoa from thawed samples has always been the main objective of semen cryopreservation technology. The goal is to minimize intracellular ice crystal formation while maintaining maximum cell dehydration. For this, a variety of cryoprotectant have been used, both glycerol- and non-glycerol-based. Cryoprotectants reduce a solution's freezing point and stop ice from accumulating inside spermatozoa by increasing the amount of salts and solutes in the liquid phase of the sample. The human uterus is regarded to be the best natural incubator for inseminated semen samples. As a result, most doctors advocate for inseminating semen samples as soon as they thaw. This is because they are worried that if the sample is kept in vitro for a long time, the sperm can become non-functional. In addition to performing computer assisted semen analysis of the frozen sperm sample after thawing and estimating their cryosurvival, the objectives of this prospective study were to evaluate the progressive motility recovery rate of the frozen spermatozoa 20 and 40 minutes post-thawing. Therefore, the goal of this study was to reassess the optimal time to conceive following the thawing of the semen sample (16).

Objective:

1.To compare the sperm motility before semen cryopreservation
2.To compare the methods of semen cryopreservation
3.To compare the methods of semen thawing
4.To compare the sperm motility after semen thawing

Statement of problem:

Non-optimal freezing effects are recognized post thaw by increased cell rupture and early stage necrosis occurring over the first few hours post-thaw. If cooling rates are too slow, prolonged exposure to multicolour levels of the freeze concentrated solutes results in cell toxicity (solution effects).

The viability and motility of frozen-thawed sperm is usually lower than that of fresh sperm. For some men, or for some semen samples of any man, cryopreservation and thawing may adversely affect the sperm so that most or all of them may be non-moving or dead.

Hypothesis:

Sperm cryopreservation has been used widely sience the 1970 to treat to treat couples with infertility. Sperm freezing is principally used to store sperm in patients undergoing concern there by and play a vital role in treating couples with infertility.

This finding supports the hypothesis that the freezing-thawing procedure cause more damage Motility some studies suggest that vitrification may result in higher post-thaw motility compared to slow freezing DNA Fragmentation: Cryopreservation can increase sperm DNA fragmentation. Freeze-thawing can impair plasma membrane integrity, mitochondrial activity, and ATP content, affecting sperm motility Osmotic effects during cryopreservation can damage cell membranes and acrosome, further impacting sperm motility.

Research methodology:

1. The sample of semen was taken and stored at 37°C to dissolve it in a sterile container.

2. The usual semen examination was carried out following liquefaction in compliance with WHO (2021) manual of the World Health Organization.

3. Additional parameters were noted, along with the concentration and motility of the sperm.

4.The 0.7 ml of semen freezing media must be warmed and brought to room temperature (37°C) before to usage.

5. After adding equilibrated semen freezing medium drop by drop over the course of ten minutes at 370C with light shaking, we cryopreserved one milliliter of semen.

6. This suspension was loaded into a pre-labeled cryovial, and the vapor was produced using LN2. For a minimum of ten minutes, freeze it.

7. The vials were placed in a suitable canister and left to defrost for ten minutes after being dipped in LN2 (-1960C). Information on the location and storage of the cryopreserved vial is included in the freezing record book.(17)

Slow freezing: The goal of slow freezing is to maintain the chemical potential of the cell water close to equilibrium with the chemical potential of the water in the partially frozen external medium by using the fastest cooling rate that will produce sufficient osmotic dehydration. (18) Morris (1970) presented a two-factor theory: cells chilled too slowly perish from prolonged exposure to concentrated solutions formed during the stepwise conversion of water to ice, and cells cooled too quickly perish from intracellular ice production. The equilibrium between these two forces causes survival vs. cooling rate graphs to take the shape of an inverted "U." The slow freezing method, which is currently frequently used for traditional sperm cryopreservation, involves progressively cooling the cells in two or three phases over the course of two to four hours.(19) His slow freezing is not the best method for cryopreserving spermatozoa in singles or small quantities.(20)

Rapid Freezing:

Sherman was the one who first suggested rapid freezing.(21)This method necessitates immersion in liquid nitrogen at -196°C for 8–10 minutes and direct contact between the crovial and the nitrogen vapors. There is a temperature gradient inside nitrogen vapours that depends on the volume and distance of the liquid below. Initially, the sample is combined drop wise with an equivalent volume of cold cryoprotectant; the

mixture is then filled into the straws and incubated for ten minutes at 4°C. After that, the straws are positioned 15–20 cm above the liquid nitrogen (−80°C) for duration of 15 minutes; following that, the straws are submerged in liquid nitrogen. (22)The cryopreservation of individual or small quantities of them is commonly accomplished by fast freezing followed by the addition of CPAs that contain highly permeate compounds. For this approach, the carrier must be submerged in nitrogen liquid at a temperature of −196°C and come into direct contact with nitrogen vapor for 8–10 minutes.9. Nonetheless, compared to slow freezing, quick freezing results in better post-thaw motility and survival rates.(23)It is highly difficult to totally extract these low-molecular-weight compounds from spermatozoa after thawing, and this approach does not fully address the issue of ice crystal formation and recrystallization. (24)

Sperm thawing:

To obtain and prepare frozen sperm samples for use in ART procedures, a sperm thawing method is utilized.

1. Remove the vial or straw from the appropriate canister after verifying the patient's identity and other pertinent information.
2. Take off the tissue paper's top layer.
3. Submerge it in water for five minutes with a laminar air flow.
4. Use tissue paper to wipe the vial.
5. For a duration of twenty to thirty minutes, keep the veil in the digital incubator at 37 0C. 6. Combine well and analyze the semen

Keep track of the motility and number of sperm.
Should it be necessary, prepare the semen.
9. Submit a report to the consultant, etc.(25)
Thawing the cryovial with spermatozoa was done in a water bath that had been heated to 37 °C beforehand. Following a 5-minute incubation period, the straw contents were moved into a centrifuge tube and cleaned using room-temperature-warm sperm preparation liquid.(26)

Analysis:

Humans spermatozoa cryopreserved has been linked to a higher risk of sperm DNA fragmentation.(27)It was reported that thawing of human sperm at 37°C and 40°C resulted in a significant increase in DNA fragmentation in different sperm subsets .(28)
The formation and reactivity of oxygen radicals may increase when the thawing temperature rises over 37°C, which could be damaging to sperm survival.(29)
When compared to fresh semen, the proportion of sperm motility in cryopreserved semen was much lower, at over 74%. Fresh semen diluted with extender and allowed to equilibrate for 15 minutes did not affect the fresh semen's sperm motility metrics.(30)

Conclusion:

In this review of literature, we found a considerable decline in sperm motility after cryopreservation and observed after thawing, sperm motility was 37.66 %. In comparison to pre cryopreservation, sperm motility was 61.82 %. The reason for this may be the type of cryopreservents used. Age may be the other factor that can cause a reduction in semen parameters. Non-malignant diseases like diabetes can also be one of the reasons for the decline in semen parameters. However, cryopreservation should be offered to patients with several malignant or nonmalignant diseases and fertility preservation. Sperm motility decrease depend upon the procedure followed by researchers someone doing with slow freezing techniques someone rapid freezing according to that survival of sperm is different on every techniques. In this reviews find that the survival rates better in slow freezing compare with vitrification.

Recommendation:

Further studies are required to confirm the present findings in large number of studies, along with the determination of underlying causes of decrease in semen parameters after cryopreservation and after the thawing of semen sample.

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