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Research Article

Cryopreserved Human Sperm Motility Enhancement Via Temporary Energy Restriction

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Abstract

As male fertility rates have declined in recent years, this study explores potential interventions to improve sperm motility. Determination of sperm motility at each time interval following nutrient introduction enabled assessment of the effect of temporary energy restriction on post-thaw motility in cryopreserved sperm samples. These observations suggest that the timing of nutrient availability plays a critical role in modulating sperm function post-thaw. The findings of this study demonstrate that TER followed by controlled nutrient introduction was associated with improved sperm motility in samples diagnosed with asthenozoospermia.

Methods: A total of 130 semen samples were assessed prior to cryopreservation. Following thawing, cryoprotectants were removed, and baseline post-thaw motility was evaluated. The samples were divided into two groups: post-thaw samples without temporary energy restriction (TER) (n = 65) and post-thaw samples subjected to TER (n = 65). The samples were subsequently exposed to GMOPS medium at a volume of 0.5 mL and incubated over defined time intervals. Sperm motility was assessed at each interval to evaluate the effect of TER on post-thaw motility. The study was conducted in accordance with ICMR guidelines.

Results: Sperm motility in the asthenozoospermic group increased following exposure to 0.5 mL of GMOPS medium across different incubation time intervals. The highest motility of 34.2% was observed at 30 minutes following TER treatment. Although motility decreased with extended incubation, the values still remained higher than those recorded immediately after media was added post-thaw.

Conclusion: Temporary energy restriction was associated with improved sperm motility, particularly at earlier time points. The findings indicate that initial nutrient deprivation followed by timed exposure to media resulted in measurable enhancement of motility. These results suggest that semen samples may benefit from this intervention prior to use in assisted reproductive procedures.

Keywords: Cryopreservation; Asthenozoospermia; Sperm Motility; Temporary Energy Restriction; Sperm Metabolism

Introduction

Cryopreservation is an integral component of assisted reproductive technology (ART). The freeze-thaw process, however, can adversely affect sperm motility and functional capacity. Alterations occurring during cryopreservation may compromise membrane integrity, mitochondrial activity, and overall cellular function, resulting in reduced progressive motility [1-3]. Structural and metabolic disturbances following freezing and thawing have also been associated with changes in sperm viability and fertilization competence [2,4]. As motility is a critical parameter in semen evaluation, approaches aimed at improving post-thaw performance are of clinical importance.

Sperm activity is closely linked to metabolic regulation, capacitation processes, and energy balance [5]. Modulation of post-thaw conditions may therefore influence the functional recovery of spermatozoa. In this context, temporary energy restriction or transient sperm starvation has been proposed as a strategy capable of enhancing ART-related outcomes and improving sperm functional competence [5,6]. Experimental evidence suggests that controlled metabolic modulation can preserve motility without prematurely triggering capacitation-associated events [7]. Furthermore, optimization of post-thaw sperm function has been shown to improve assisted reproduction outcomes in certain models [9].

In light of these findings, investigation of metabolic regulation strategies following cryopreservation represents a rational and biologically relevant approach for improving sperm quality in clinical settings.

Objective of the Study

The aim of this study was to investigate sperm motility in cryopreserved semen samples after applying TER post-thawing. The objective was to elevate sperm motility by introducing media at specified time intervals.

Materials and Methods

Sperm capacitation is a physiological process that occurs as spermatozoa traverse the female reproductive tract. During this process, seminal plasma components, including prostaglandins, are removed, enabling the sperm to move and acquire fertilizing capacity. Only capacitated sperm are able to progress toward the ovum and initiate fertilization; therefore, the importance of motility is clearly understood.

The study utilized anonymized clinical semen samples from men diagnosed with asthenozoospermia, characterized by reduced sperm motility parameters. Samples were collected as part of routine diagnostic procedures. Written informed consent was obtained from all participants prior to collection, and the study was conducted with institutional authorization in accordance with established clinical protocols.

All samples were maintained at 37°C under identical handling conditions, and the duration of energy restriction was strictly standardized across all experimental replicates.

Comprehensive macroscopic and microscopic analyses were performed prior to cryopreservation. Macroscopic evaluation included assessment of semen volume, colour, pH, viscosity, and liquefaction time. Microscopic examination was performed using a Makler chamber and involved determination of sperm concentration, motility, morphology, vitality, aggregation, and agglutination according to the World Health Organization (WHO) manual.

Prior to cryopreservation, 130 fresh semen samples were collected, and a small portion of each sample was assessed for motility immediately after collection and subsequently at 30, 45, 60, 75, and 90 minutes without any intervention. These samples were observed solely for motility changes over time. The remaining portion of each sample, after aliquoting for motility assessment, underwent cryopreservation following a standard procedure, in which an equal volume of cryoprotectant (1:1 ratio) was added to the semen sample, mixed thoroughly, and then transferred into a cryovial for storage at −196 °C using Repro Sperm cryopreservation medium.

Cryopreserved samples were thawed by removing the cryovial from −196 °C storage and allowing it to reach room temperature followed by evaluation for initial survival and motility. Cryoprotectants were removed by washing the samples with Vitro med GMOPS-buffered Human Tubal Fluid (HTF) containing Human Serum Albumin (HSA). All media used were endotoxin-tested and Mouse Embryo Assay (MEA)-tested. GMOPS (Gamete Medium Optimized for Physiological Solutions) provides a balanced buffering system that maintains stable pH and osmolarity during gamete handling outside the incubator, offering enhanced protection against pH fluctuations.

For cryoprotectant removal, 1 mL of GMOPS medium, pre-equilibrated at 37 °C, was added to a sterile centrifuge tube. Using a sterile pipette, 1 mL of semen sample containing cryoprotectants was mixed thoroughly and centrifuged at 1200 rpm for 10 minutes. The supernatant was discarded to eliminate residual cryoprotectants, leaving only the sperm pellet.

Each donor sample was initially divided into five cryovials for assessments at 30, 45, 60, 75, and 90 minutes post-thaw. Samples were assigned unique identifiers and randomly allocated into two groups using a computer-generated randomization sequence (Python, version 3.13) through simple randomization without

replacement: (i) thawed samples subjected to temporary energy restriction (TER) (n = 65) and (ii) thawed samples not subjected to TER (n = 65).

For the TER group, GMOPS medium was added to the pellet after 30, 45, 60, 75, and 90 minutes of restriction, and sperm motility was assessed at each time point following nutrient introduction. In contrast, for the non-TER group, medium was added immediately after centrifugation, and motility was observed at corresponding intervals of 30, 45, 60, 75, and 90 minutes.

At each time interval, 0.5 mL of GMOPS medium was added to the centrifuge tubes. Using a sterile pipette, 10 µL of the semen sample was added to the Makler chamber to assess sperm motility, which was counted manually according to the WHO laboratory manual for the examination and processing of human semen. Motility was recorded at each time point for subsequent analysis.

This protocol enabled evaluation of the effect of temporary energy restriction on post-thaw motility in cryopreserved sperm samples, as well as baseline motility patterns in fresh samples.

Statistical analysis was performed using Python (version 3.13) with the statsmodels package. Data were expressed as mean ± standard deviation. A two-way analysis of variance (ANOVA) was conducted to evaluate the effects of treatment group (Before cryopreservation, Without TER, and With TER), time (Sets 1–5), and their interaction on sperm motility. Statistical significance was determined at $p < 0.05$.

Results

Time (min)	Before Cryo-preservation (n = 130) Mean ± SD	Without TER (n = 65) Mean ± SD	With TER (n = 65) Mean ± SD
30	38.7 ± 1.9	30.6 ± 0.6	34.2 ± 0.5
45	34.5 ± 1.0	26.4 ± 0.5	31.3 ± 0.4
60	33.2 ± 0.8	23.2 ± 0.4	27.8 ± 0.3
75	33.0 ± 0.7	21.1 ± 0.3	24.3 ± 0.3
90	33.0 ± 0.6	19.8 ± 0.3	26.1 ± 0.3

Table 1: Mean sperm motility.

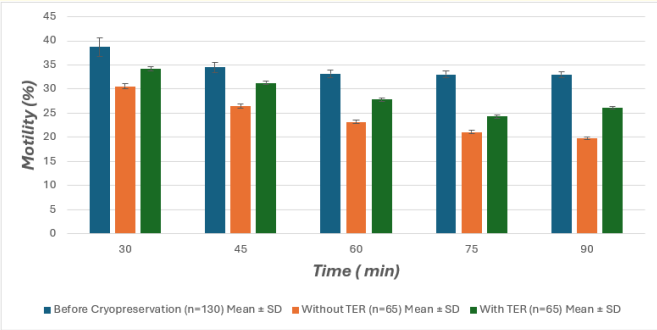

Figure 1: Sperm motility (%) in asthenozoospermic samples before cryopreservation and post-thaw under TER and non-TER conditions across defined time intervals. Values are presented as mean ± standard deviation.

A total of 130 donor samples were included in the study. Prior to cryopreservation, the mean sperm motility was 38.7%. Following thawing, baseline motility was comparable between groups prior to nutrient intervention, although slightly higher values were observed in the TER group. Mean sperm motility values before cryopreservation and at 30, 45, 60, 75, and 90 minutes post-thaw for samples with and without temporary energy restriction (TER) are presented in Table 1.

A two-way analysis of variance (ANOVA) demonstrated a significant effect of time on sperm motility ($p < 0.001$), indicating variation across incubation intervals. A significant interaction between time and treatment group was also observed ($p < 0.05$), suggesting that the effect of TER on motility was dependent on the incubation period. Although the overall main effect of treatment group was not statistically significant, the interaction suggests that TER influences motility differently across the observed time points.

As shown in Figure 1, sperm motility was consistently higher in the TER group compared to the non-TER group at all observed time points. Although motility declined progressively over time in both groups, the reduction was less pronounced in samples subjected to TER.

Discussion

The findings indicate that samples subjected to temporary energy restriction (TER) demonstrated consistently higher motility compared to those receiving immediate nutrient supplementation.

This trend was particularly evident at earlier time points, followed by a gradual decline in motility with extended incubation in both groups.

These observations suggest that the timing of nutrient availability plays a critical role in modulating sperm function post-thaw. Previous studies investigating transient sperm starvation have demonstrated that controlled metabolic restriction can improve assisted reproductive outcomes and modulate sperm functional responses [5]. One possible explanation is that temporary restriction may allow sperm cells to undergo metabolic adaptation, thereby enhancing their responsiveness to subsequent nutrient supplementation. Evidence indicates that sperm motility and function are closely linked to mitochondrial activity and metabolic regulation [1,8]. This controlled introduction could therefore optimise energy utilisation, leading to improved motility outcomes.

The gradual reduction in motility observed over time may reflect the natural limitations of sperm viability during extended incubation, particularly in cryopreserved samples, where mitochondrial and membrane integrity may already be compromised [1,2]. The comparative difference between the TER and non-TER groups underscores the potential of metabolic modulation strategies in improving cryopreservation outcomes. Cryopreservation has been consistently associated with reductions in motility and functional capacity [1,2,4], and approaches that enhance post-thaw recovery are therefore clinically relevant.

While immediate nutrient supplementation did result in measurable motility values, the motility observed in the TER group remained comparatively higher across the observed time intervals. This suggests that nutrient availability alone may not be sufficient; rather, the sequence and timing of metabolic interventions appear important for maximising functional recovery. Similar findings have been reported in studies examining metabolic regulation and capacitation-related processes in frozen-thawed semen [9]. The underlying mechanisms behind the effect of cryopreservation on sperm parameters are not completely understood. Genes and protein expression, mRNA stability and epigenetic content of spermatozoa are thought to be modulated during the freeze-thaw process.

It is important to acknowledge that this study was limited to motility assessment over a 90-minute observation period. Future research should explore whether the observed improvements in motility translate into enhanced fertilisation potential and clinical outcomes. Additionally, investigation of underlying physiological mechanisms, including mitochondrial function and metabolic adaptations, may provide further insight into the biological basis of the observed effects [1,6].

Conclusion

The findings of this study demonstrate that temporary energy restriction followed by controlled nutrient introduction was associated with higher sperm motility, particularly in a time-dependent manner. In the TER group, the highest motility was observed at 30 minutes following nutrient introduction, after which motility values gradually declined with extended incubation. In contrast, samples that did not undergo temporary energy restriction showed comparatively lower motility across all observed time intervals. These observations indicate that implementing TER prior to nutrient supplementation enhances motility outcomes in cryopreserved asthenozoospermic samples.

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