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Original Article

Effects of Pentoxifylline Timing and Storage Temperature on Human Sperm Motility, Morphology, and Viability

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Abstract

Objective: Short-term liquid storage of human sperm is frequently required in assisted reproductive technologies, yet storage-related declines in sperm quality remain a challenge. Pentoxifylline (PTX) has been used to enhance sperm motility, but the optimal timing of PTX administration in relation to storage temperature is unclear.

Materials and methods: In this experimental laboratory study, semen samples from 30 normozoospermic men were divided into six paired aliquots and stored for 24 hours at room temperature or 4 °C. PTX (3 mmol/L) was administered either before storage, after storage, or not at all. Sperm motility, viability, and morphology were assessed using standard light microscopy.

Results: Significant differences were observed across groups for sperm motility parameters and abnormal morphology ($p < 0.05$), whereas sperm viability remained comparable. Delayed PTX administration after storage at room temperature was associated with the poorest motility and morphological outcomes. A composite rank-based analysis identified pre-PTX storage at room temperature as the most favorable overall strategy, with pre-PTX storage at 4 °C ranking second; however, direct comparison between these two approaches revealed no statistically significant differences.

Conclusion: Sperm quality during 24-hour liquid storage is influenced by both storage temperature and, more critically, the timing of PTX administration. Pre-storage PTX may serve as a protective adjunct in short-term sperm storage, while delayed administration confers limited benefit. Further studies are warranted to refine its clinical application.

Keywords: Pentoxifylline; Sperm Motility; Sperm Morphology; Sperm Viability; Semen Preservation; Assisted Reproductive Techniques

Introduction

Short-term sperm storage is routinely required in assisted reproductive technologies (ART) and laboratory settings to accommodate scheduling constraints, transport, or pre-processing delays. Maintaining optimal sperm motility and morphology during storage is essential to preserve fertilizing potential, as these parameters are strong predictors of ART success and embryo development (1, 2). However, even brief storage periods can compromise sperm quality, resulting in reduced fertilization rates (3). The ability to identify ideal storage conditions that balance sperm preservation with convenience

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remains a key challenge in clinical and research applications of reproductive medicine (4).

Sperm storage temperature and duration significantly affect motility, viability, and DNA integrity (5, 6). Studies show that storage at both room temperature and low temperatures (4 °C) leads to a decline in motility and membrane stability after 24 hours (7). This deterioration is primarily due to oxidative stress, ATP depletion, and membrane lipid peroxidation (8). Low temperatures slow metabolism but may induce cold shock, altering membrane fluidity and impairing mitochondrial function (9). Conversely, room temperature storage preserves enzyme activity but accelerates reactive oxygen species (ROS) generation and structural damage (6). Therefore, oxidative stress and energy depletion represent core mechanisms underlying sperm deterioration during short-term storage.

Human spermatozoa are highly vulnerable to oxidative injury because they possess limited antioxidant defenses and abundant polyunsaturated fatty acids (10). Pentoxifylline (PTX), a phosphodiesterase inhibitor, increases intracellular cyclic AMP, enhances mitochondrial activity, and reduces oxidative stress (11, 12). Numerous studies have demonstrated that PTX can improve sperm motility and viability (13, 14), including after cryopreservation or cold storage (15). It has also been reported to maintain sperm ultrastructure following vitrification and reduce ROS-mediated damage (16). However, its effects are dose-dependent, and excessive PTX exposure may induce premature acrosome reactions or DNA fragmentation (17, 18). Thus, while PTX shows promise as an additive to semen extenders for short-term storage, its optimal concentration and safety profile require further evaluation in human sperm held at 4 °C or room temperature for 24 hours.

Despite extensive research on the effects of PTX on sperm motility and viability, the optimal timing of its application relative to storage conditions remains unclear. Most studies have either applied PTX immediately before sperm analysis or incorporated it into cryopreservation protocols, leaving uncertainty as to whether PTX acts more effectively as a protective additive prior to storage or as a stimulatory agent following storage (9, 12, 15). Moreover, few investigations have assessed how storage temperature, whether room temperature or refrigeration at 4 °C, modulates the pharmacodynamic effects of PTX on sperm physiology. While PTX has been shown to enhance motility and reduce oxidative damage in various experimental settings (11, 14, 17), there is a lack of comprehensive data integrating timing, temperature, and duration of storage as interacting factors influencing motility, morphology, viability, and ultrastructural preservation. This gap underscores the need for systematic evaluation of PTX's dual role as both a preventive antioxidant and a post-storage motility activator under controlled thermal conditions. Given these uncertainties, the present study was designed to systematically evaluate the combined effects of PTX timing (pre-storage vs. post-storage) and temperature (room temperature vs. 4 °C) on human sperm motility, morphology, and viability after 24 hours of storage.

Materials and methods

Study Design and Semen Samples: This experimental laboratory study was conducted on semen samples obtained from 30 normospermic men. Following liquefaction, each semen sample was divided into six equal aliquots to allow paired comparisons across experimental conditions. All aliquots were initially incubated at room temperature for 30 minutes and subsequently diluted in Ham's F-10 medium before undergoing a 24-hour storage period.

Experimental Conditions: Aliquots were allocated to six experimental conditions defined by storage temperature and timing of PTX exposure. Storage was performed either at room temperature or at 4 °C. PTX was administered at a final concentration of 3 mmol/L according to the following protocols: Aliquot 1, PTX added prior to storage at room temperature; Aliquot 2, PTX added prior to storage at 4 °C; Aliquot 3, PTX added after a 24-hour storage period at room temperature; and Aliquot 4, PTX added after a 24-hour storage period at 4 °C. Two aliquots served as controls and received no PTX: Aliquot 5, stored at room temperature, and Aliquot 6, stored at 4 °C. This experimental design resulted in a 2 × 3 factorial structure comprising storage temperature (room temperature vs 4 °C) and intervention strategy (PTX before storage, PTX after storage, or no intervention).

Outcomes: The primary outcome of the study was the percentage of motile sperms. Secondary outcomes included motility sub-groups, sperm viability, overall abnormal morphology, specific morphological defects such as loose head, bent neck, and coiled tail.

Assessment of Sperm Parameters: Following the

storage period and intervention procedures, all aliquots were evaluated using light microscopy to assess sperm motility, viability, and morphology in accordance with standard laboratory protocols. Sperm motility was classified into immotile sperm (Motility I), non-progressively motile sperm (Motility II), and progressively motile sperm (Motility III + IV), and results were expressed as percentages. Total motility was calculated as the sum of non-progressive and progressive motility. Sperm viability was determined and reported as the percentage of live spermatozoa. Morphological abnormalities were recorded as the percentage of abnormal forms, including specific defects such as loose head, bent neck, and coiled tail.

Statistical Analysis: Continuous variables were assessed for distributional normality using visual inspection of histograms and the Shapiro–Wilk test. As all sperm quality parameters demonstrated non-normal distributions, results are presented as medians with interquartile ranges, and non-parametric statistical methods were applied throughout. Comparisons of sperm morphology, viability, and motility parameters across the six storage and intervention groups (defined by PTX exposure timing and storage temperature) were performed using the Kruskal–Wallis rank sum test. For outcomes showing statistically significant global differences, post-hoc pairwise comparisons were conducted using Dunn's test with adjustment for multiple testing by the Benjamini–Hochberg method. To identify the optimal storage and intervention method based on overall sperm quality, a non-parametric rank-based analysis was performed using three non-overlapping indicators: total motility, viability, and abnormal morphology. For each group, median values of these variables were calculated. Groups were then ranked for each outcome according to biological favorability (higher total motility and viability, and lower abnormal morphology), and an overall mean rank was derived by averaging the individual ranks. Lower mean rank values indicated superior overall sperm quality. Differences in overall ranking between groups were assessed using the Kruskal–Wallis test. In addition, pairwise comparisons between the pre-PTX room temperature and pre-PTX 4 °C groups (first and second overall rank) were performed for all sperm quality parameters using the Mann–Whitney U test, with results presented as medians and interquartile ranges. All statistical analyses were conducted using R software (version 4.5.1). A two-sided p value < 0.05 was considered statistically significant.

Results

A total of 30 normospermic semen samples were analyzed under six different storage and intervention conditions, with all outcomes expressed as medians and interquartile ranges due to non-normal data distribution (Table 1). Significant differences were observed across groups for sperm motility parameters and abnormal morphology, while viability did not differ significantly between conditions.

The percentage of immotile sperm (Motility I) differed significantly among the six experimental conditions ($p = 0.009$), with higher median values observed in the pre-storage PTX at room temperature group compared with lower values in post-storage PTX and control conditions (Table 1). Non-progressive motility (Motility II) also showed a significant overall difference across groups ($p = 0.002$), with the no-PTX 4 °C group demonstrating the highest median values compared with near-zero medians in most PTX-treated groups (Table 1). Progressive motility (Motility III + IV) varied significantly between groups ($p = 0.031$), although median values remained low across all conditions, with slightly higher upper quartile values observed in the no-PTX 4 °C and pre-PTX room temperature groups (Table 1).

Total motility demonstrated a statistically significant difference across storage and intervention methods ($p = 0.001$), with the no-PTX 4 °C group showing the highest median total motility compared with PTX-treated groups, particularly those receiving PTX after storage (Table 1). In contrast, sperm viability did not differ significantly across experimental conditions, with median viability ranging from 50.3% to 75.6% and overlapping interquartile ranges across all groups ($p = 0.619$) (Table 1).

Overall abnormal sperm morphology differed significantly among groups ($p = 0.002$), with higher median abnormal morphology observed in the post-storage PTX at room temperature and no-PTX room temperature groups compared with lower values in the pre-storage PTX groups.

Among specific morphological defects, the proportion of sperm with coiled tails varied significantly across conditions ($p = 0.012$), with higher medians observed in the post-storage PTX at room temperature and no-PTX room temperature groups compared with lower values in the post-storage PTX at 4 °C group (Table 1).

Table 1: Comparison of sperm morphology, viability, and motility parameters across storage and intervention methods

Outcome	Aliquot 1	Aliquot 2	Aliquot 3	Aliquot 4	Aliquot 5	Aliquot 6	p-value[1]
	N = 30	N = 30	N = 30	N = 30	N = 30	N = 30	
Motility I	3.3 (0.0, 6.1)	0.0 (0.0, 6.8)	0.0 (0.0, 0.8)	0.0 (0.0, 2.3)	0.3 (0.0, 2.5)	2.0 (0.0, 5.6)	0.009
Motility II	0.0 (0.0, 8.6)	0.0 (0.0, 3.7)	0.0 (0.0, 0.0)	0.0 (0.0, 1.6)	0.0 (0.0, 0.0)	2.2 (0.0, 4.6)	0.002
Motility III + IV	0.0 (0.0, 1.6)	0.0 (0.0, 0.0)	0.0 (0.0, 0.0)	0.0 (0.0, 0.0)	0.0 (0.0, 0.0)	0.0 (0.0, 1.3)	0.031
Total motility	3.7 (0.0, 22.7)	0.0 (0.0, 7.4)	0.0 (0.0, 0.8)	0.0 (0.0, 3.1)	0.3 (0.0, 4.8)	5.0 (1.0, 12.5)	0.001
Viability	75.6 (28.6, 87.5)	67.9 (16.0, 81.3)	50.3 (9.5, 80.6)	58.8 (26.0, 86.7)	62.2 (29.1, 82.6)	52.5 (27.5, 84.6)	0.619
Abnormal morphology	72.0 (63.9, 79.0)	70.0 (57.1, 82.1)	83.3 (78.2, 92.9)	78.6 (63.9, 89.2)	84.4 (77.3, 90.0)	81.3 (70.8, 89.3)	0.002
Loose head	6.3 (0.0, 10.0)	8.7 (0.0, 16.7)	8.7 (5.0, 12.5)	8.8 (0.0, 18.4)	7.1 (0.0, 12.1)	8.3 (6.3, 13.3)	0.623
Bent neck	9.7 (4.4, 14.3)	10.3 (3.2, 17.4)	10.0 (2.7, 16.0)	8.3 (4.0, 18.4)	8.8 (4.6, 15.0)	12.1 (3.4, 16.7)	0.963
Coiled tail	21.7 (10.9, 31.4)	18.8 (4.4, 30.0)	35.0 (14.3, 55.0)	14.8 (2.3, 27.5)	36.4 (11.5, 50.0)	17.6 (10.2, 32.8)	0.012

[1]Kruskal-Wallis rank sum test

†Data are presented as median (interquartile range).

‡Aliquot 1: pentoxifylline (PTX) added prior to storage at room temperature; Aliquot 2: PTX added prior to storage at 4 °C; Aliquot 3: PTX added after 24-hour storage at room temperature; Aliquot 4: PTX added after 24-hour storage at 4 °C; Aliquot 5: no PTX, stored at room temperature; Aliquot 6: no PTX, stored at 4 °C. PTX was used at a final concentration of 3 mmol/L.

Table 2: Significant pairwise differences in sperm quality outcomes following post-hoc analysis

Outcome	Comparison	Direction	Adjusted p-value
Motility I	Aliquot 3 vs. Aliquot 6	Higher in Aliquot 6	0.016
Motility II	Aliquot 1 vs. Aliquot 3	Higher in Aliquot 1	0.012
Motility II	Aliquot 3 vs. Aliquot 6	Higher in Aliquot 6	0.002
Total motility	Aliquot 1 vs. Aliquot 3	Higher in Aliquot 1	0.018
Total motility	Aliquot 3 vs. Aliquot 6	Higher in Aliquot 6	<0.001
Abnormal morphology	Aliquot 1 vs. Aliquot 3	Lower in Aliquot 1	0.012
Abnormal morphology	Aliquot 1 vs. Aliquot 5	Lower in Aliquot 1	0.021
Abnormal morphology	Aliquot 2 vs. Aliquot 3	Lower in Aliquot 2	0.043
Coiled tail	Aliquot 3 vs. Aliquot 4	Lower in Aliquot 4	0.028
Coiled tail	Aliquot 4 vs. Aliquot 5	Lower in Aliquot 4	0.044

Aliquot 1: pentoxifylline (PTX) added prior to storage at room temperature; Aliquot 2: PTX added prior to storage at 4 °C; Aliquot 3: PTX added after 24-hour storage at room temperature; Aliquot 4: PTX added after 24-hour storage at 4 °C; Aliquot 5: no PTX, stored at room temperature; Aliquot 6: no PTX, stored at 4 °C.

In contrast, the frequency of loose head abnormalities did not differ significantly between groups (p = 0.623), nor did the prevalence of bent neck defects, which remained comparable across all experimental conditions (p = 0.963) (Table 1).

Post-hoc pairwise comparisons were performed for outcomes demonstrating significant overall differences to identify specific contrasts between storage and intervention conditions. For immotile sperm (Motility I), a significantly higher proportion was observed in the no-PTX 4 °C group compared with the post-storage PTX at room temperature group (adjusted p = 0.016), indicating increased immotility under cold storage without pharmacological intervention relative to delayed PTX exposure (Table 2).

For non-progressive motility (Motility II), significant differences were identified between multiple groups. The pre-storage PTX at room temperature group exhibited higher Motility II compared with the post-storage PTX at room temperature group (adjusted p = 0.012), while the no-PTX 4 °C group also demonstrated significantly higher Motility II than the post-storage PTX at room temperature group (adjusted p = 0.002) (Table 2). Total motility differed significantly between key intervention strategies. Total motility was higher in the pre-storage PTX at room temperature group compared with the post-storage PTX at room temperature group (adjusted p = 0.018), and was markedly higher in the no-PTX 4 °C group compared with the post-storage PTX at room temperature group (adjusted p < 0.001) (Table 2).

Significant differences were also observed for abnormal sperm morphology. The proportion of abnormal forms was significantly lower in the pre-storage PTX at room temperature group compared with both the post-storage PTX at room temperature group (adjusted p = 0.012) and the no-PTX room temperature group (adjusted p = 0.021). In addition, abnormal morphology was lower in the pre-storage PTX at 4 °C group compared with the post-storage PTX at room temperature group (adjusted p = 0.043) (Table 2). Regarding specific morphological defects, significant pairwise differences were identified for coiled tail abnormalities.

The post-storage PTX at 4 °C group exhibited a significantly lower proportion of coiled tails compared with both the post-storage PTX at room temperature group (adjusted p = 0.028) and the no-PTX room temperature group (adjusted p = 0.044) (Table 2).

A non-parametric rank-based analysis integrating total motility, sperm viability, and abnormal morphology was performed to identify the optimal storage and intervention strategy. Clear differences in overall sperm quality were observed across the six experimental conditions, as reflected by distinct mean rank values (Table 3). Pre-storage PTX exposure at room temperature demonstrated the most favorable overall profile, achieving the lowest mean rank of 1.7, driven by high rankings for total motility and abnormal morphology and the top rank for viability. Pre-storage PTX at 4 °C ranked second overall with a mean rank of 2.7, reflecting the best performance in terms of abnormal morphology and a favorable viability rank, despite a less favorable ranking for total motility.

The no-PTX 4 °C group achieved an intermediate overall ranking with a mean rank of 3.3, characterized by the highest rank for total motility but poorer performance in viability and abnormal morphology. Both post-storage PTX at 4 °C and no-PTX room temperature groups demonstrated similar overall rankings, each with a mean rank of 4.0, indicating moderate sperm quality across the combined outcomes. In contrast, post-storage PTX at room temperature ranked lowest among all groups, with a mean rank of 5.3, reflecting consistently poor performance across total motility, viability, and abnormal morphology (Table 3).

Table 3: Overall non-parametric ranking of storage and intervention methods based on total motility, viability, and abnormal morphology

Aliquot	Total Motility Rank	Viability Rank	Abnormal Morphology Rank	Mean Rank
Aliquot 1	2	1	2	1.7
Aliquot 2	5	2	1	2.7
Aliquot 6	1	5	4	3.3
Aliquot 4	5	4	3	4.0
Aliquot 5	3	3	6	4.0
Aliquot 3	5	6	5	5.3

Aliquot 1: pentoxifylline (PTX) added prior to storage at room temperature; Aliquot 2: PTX added prior to storage at 4 °C; Aliquot 3: PTX added after 24-hour storage at room temperature; Aliquot 4: PTX added after 24-hour storage at 4 °C; Aliquot 5: no PTX, stored at room temperature; Aliquot 6: no PTX, stored at 4 °C.

Direct pairwise comparison between pre-storage PTX at room temperature and pre-storage PTX at 4 °C was conducted to further evaluate differences between the two highest-ranked intervention strategies.

Table 4: Comparison of sperm quality parameters between pre-PTX storage at room temperature and 4 °C

Outcome	Aliquot 1	Aliquot 2	p-value
Motility grade I	3.3 (0.0–6.0)	0.0 (0.0–6.1)	0.456
Motility grade II	0.0 (0.0–8.3)	0.0 (0.0–3.6)	0.404
Motility grade III + IV	0.0 (0.0–1.5)	0.0 (0.0–0.0)	0.095
Total motility	3.7 (0.0–22.3)	0.0 (0.0–7.3)	0.215
Viability	75.6 (28.7–87.4)	67.9 (18.2–80.9)	0.473
Abnormal morphology	72.0 (63.9–79.0)	70.0 (61.6–81.1)	0.838
Loose head	6.2 (0.0–10.0)	8.7 (1.1–16.7)	0.371
Bent neck	9.7 (4.4–14.3)	10.3 (4.0–17.0)	0.398
Coiled tail	21.7 (10.9–31.4)	18.8 (6.7–28.9)	0.412

Aliquot 1: pentoxifylline (PTX) added prior to storage at room temperature; Aliquot 2: PTX added prior to storage at 4 °C.

No statistically significant differences were observed between these groups across any of the assessed sperm quality parameters, indicating comparable performance of pre-PTX administration at both storage temperatures (Table 4).

Discussion

This study shows that sperm quality during 24-hour storage is strongly influenced by both storage temperature and the timing of PTX administration. Although, Sperm viability remained largely unaffected across all conditions, delayed PTX exposure at room temperature resulted in the poorest motility and morphology, whereas pre-storage PTX administration produced more favorable outcomes. A composite rank-based analysis identified pre-PTX storage at room temperature as the optimal overall strategy, while post-PTX exposure at room temperature ranked lowest. Although pre-PTX storage at room temperature demonstrated a better overall rank than pre-PTX storage at 4 °C, direct comparison revealed no statistically significant differences between these two methods.

Based on the results of this study, the timing of PTX exposure appears to be a critical determinant of sperm quality during short-term liquid storage, with pre-storage administration yielding more favorable motility and morphology profiles than delayed exposure. This finding is consistent with studies of refrigerated liquid storage demonstrating that PTX primarily preserves sperm motility rather than viability or global cellular integrity, supporting a parameter-specific effect (9, 19). The absence of significant differences in sperm viability across conditions aligns with prior reports indicating that PTX does not substantially influence survival-related endpoints during liquid storage, even when motility benefits are evident (9, 19). Moreover, baseline storage studies have shown minimal differences between room temperature and refrigerated conditions during the early storage period, suggesting that early pharmacologic protection may be more important than temperature alone within a 24-hour timeframe (20).

The inferior outcomes observed with delayed PTX exposure at room temperature suggest that PTX is less effective as a restorative intervention once storage-related functional impairment has occurred. This interpretation is supported by cryopreservation studies demonstrating that PTX effects are highly dependent on timing and context, with post-thaw exposure often enhancing motility through pharmacologic activation rather than cytoprotection, and pre-storage exposure showing variable or even detrimental effects at certain concentrations (21, 22). Dose-dependent data further indicate a narrow therapeutic window for PTX, where motility enhancement may coexist with subtle structural alterations, underscoring the importance of evaluating morphology alongside functional parameters (23). Collectively, these findings support the concept that, in short-term liquid storage models, PTX is most effective when administered prior to storage as a protective adjunct, while delayed administration, particularly at room temperature, confers limited benefit (9, 19, 20).

The strengths of this study include its paired within-sample experimental design, which minimized inter-individual variability, the simultaneous evaluation of storage temperature and PTX timing within a factorial framework, and the comprehensive assessment of multiple sperm quality domains using

appropriate non-parametric and rank-based analytical approaches. Several limitations should be acknowledged, including the relatively small sample size, evaluation limited to a single PTX concentration and a 24-hour storage period, and the absence of functional fertilization outcomes or molecular markers of oxidative stress. Future studies should explore dose–response effects, extend storage durations, integrate ultrastructural and oxidative stress assessments, and correlate laboratory findings with fertilization and reproductive outcomes to better define the clinical utility of PTX in sperm preservation protocols.

Conclusion

In conclusion, the current findings indicate that sperm quality during short-term liquid storage is influenced by both storage temperature and, more importantly, the timing of pentoxifylline administration. Pre-storage PTX exposure was associated with more favorable motility and morphological outcomes, whereas delayed administration after storage, particularly at room temperature, resulted in inferior sperm quality. Although pre-storage PTX at room temperature and 4 °C demonstrated comparable effectiveness, these results suggest that timely pharmacologic intervention is more critical than storage temperature alone for preserving sperm function over 24 hours. Collectively, the findings support a potential role for pre-storage PTX as a practical adjunct in short-term sperm storage, while underscoring the need for further studies before routine clinical implementation.

Conflict of Interests

Authors declare no conflict of interests.

Acknowledgments

None.

References

1. Melli B, Morini D, Spaggiari G, Falbo A, Lutzoni R, Mezzadri C, et al. P-032 Sperm parameters can predict the success of assisted reproductive technology. Single-center and retrospective analysis of assisted reproductive technology cycles from 1992 to 2020. Human Reproduction. 2022; 37(Supplement_1): deac107. 030.
2. Özcan A, Tulay P, İrez T. Investigation of optimal sperm storage conditions for short-term storage. Bangladesh Journal of Medical Science. 2024;23(4):1137-41.
3. Jackson RE, Bormann CL, Hassun PA, Rocha AM, Motta EL, Serafini PC, Smith GD. Effects of semen storage and separation techniques on sperm DNA fragmentation. Fertility and sterility. 2010;94(7):2626-30.
4. Neuman NM, Orzołek A, Steiner-Bogdaszewska Ż, Dziekońska A. Changes in the Morphology and Antioxidant Status of European Red Deer Sperm Stored in the Epididymides and in a Liquid State. Animals. 2024;14(11):1653.
5. Campbell L, Clulow J, Howe B, Upton R, Doody S, Clulow S. Efficacy of short-term cold storage prior to cryopreservation of spermatozoa in a threatened lizard. Reproduction, Fertility and Development. 2021;33(9):555-61.
6. Kameni SL, Meutchieye F, Ngoula F. Liquid storage of ram semen: associated damages and improvement. Open Journal of Animal Sciences. 2021;11(3):473-500.
7. De Ambrogi M, Ballester J, Saravia F, Caballero I, Johannisson A, Wallgren M, et al. Effect of storage in short-and long-term commercial semen extenders on the motility, plasma membrane and chromatin integrity of boar spermatozoa. international journal of andrology. 2006;29(5):543-52.
8. Palmieri M, Papale P, Della Ragione A, Quaranta G, Russo G, Russo S. In vitro antioxidant treatment of semen samples in assisted reproductive technology: Effects of Myo-inositol on Nemaspermic parameters. International journal of endocrinology. 2016;2016(1):2839041.
9. Nateghian Z, Nasr-Esfahani MH, Talaei-Khozani T, Tavalaee M, Aliabadi E. L-carnitine and pentoxifylline supplementation improves sperm viability and motility at low temperature. Int J Fertil Steril. 2023;17(1): 61-66.
10. Lu Y, Su H, Zhang J, Wang Y, Li H. Treatment of poor sperm quality and erectile dysfunction with oral pentoxifylline: a systematic review. Frontiers in Pharmacology. 2022;12:789787.
11. Shen M-R, Chiang P-H, Yang R, Hong C, Chen S-S. Pentoxifylline stimulates human sperm motility both in vitro and after oral therapy. British journal of clinical pharmacology. 1991;31(6):711-14.
12. Nabi A, Khalili MA, Talebi AR, Mangoli E, Yari N, Nottola SA, et al. In-vitro application of pentoxifylline preserved ultrastructure of spermatozoa after vitrification in asthenozoospermic patients. Urology journal. 2017;14(4):4038-43.
13. Ghasemzadeh A, Karkon-Shayan F, Yousefzadeh S, Naghavi-Behzad M, Hamdi K. Study of pentoxifylline effects on motility and viability of spermatozoa from

infertile asthenozoospermic males. Nigerian Medical Journal. 2016;57(6):324-28.
14. Ammar O, Ben Ali Gannoun M, Ajina T, Hadj Ali A, Boussabbeh M, Sallem A, et al. Pentoxifylline Enhances Sperm Quality, Reduces Oxidative Stress in Semen, and Decreases Sperm DNA Damage in Men with Asthenozoospermia Undergoing Assisted Reproductive Technology. Oxygen. 2025;5(2):8.
15. Esteves SC, Spaine DM, Cedenho AP. Effects of pentoxifylline treatment before freezing on motility, viability and acrosome status of poor quality human spermatozoa cryopreserved by the liquid nitrogen vapor method. Brazilian Journal of Medical and Biological Research. 2007;40:985-92.
16. Dong J, Yin M, Wu L, Wang T, Li M, Zhang W, et al. Pregnancy and neonatal outcomes of ICSI using pentoxifylline to identify viable spermatozoa in patients with frozen-thawed testicular spermatozoa. Frontiers in Endocrinology. 2024;15:1364285.
17. Satish M, Kumari S, Deeksha W, Abhishek S, Nitin K, Adiga SK, et al. Structure-based redesigning of pentoxifylline analogs against selective phosphodiesterases to modulate sperm functional competence for assisted reproductive technologies. Scientific reports. 2021;11(1):12293.
18. Dadgar Z, Shariatzadeh SMA, Mehranjani MS, Kheirolahi A. The therapeutic effect of co-administration of pentoxifylline and zinc in men with idiopathic infertility. Ir J Med Sci. 2023;192(1):431-39.
19. Aliabadi E, Nateghian Z, Nasr-Esfahani MH, Tavalaee M, Talaei-Khozani T. Effects of L-carnitine and pentoxifylline on long-term preservation of the human sperms: An experimental study. International Journal of Reproductive BioMedicine. 2025;22(11):871.
20. Golshan Iranpour F, Nateghian Z, Henkel R, Dashti GR. Effects of temperature and storage time on the motility, viability, DNA integrity and apoptosis of processed human spermatozoa. Andrologia. 2020;52(2):e13485.
21. Esteves SC, Sharma RK, Thomas Jr A, Agarwal A. Cryopreservation of human spermatozoa with pentoxifylline improves the post-thaw agonist-induced acrosome reaction rate. Human Reproduction (Oxford, England). 1998;13(12):3384-89.
22. Stanic P, Sonicki Z, Suchanek E. Effect of pentoxifylline on motility and membrane integrity of cryopreserved human spermatozoa. International journal of andrology. 2002;25(3):186-90.
23. Brennan AP, Holden CA. Andrology: Pentoxifylline-supplemented cryoprotectant improves human sperm motility after cryopreservation. Human reproduction. 1995;10(9):2308-12.

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