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Effects of Pentoxifylline and Trehalose on the quality of mouse epididymal sperm during liquid storage

Efectos de la pentoxifilina y la trehalosa sobre la calidad de los espermatozoides epididimarios de ratón durante el almacenamiento en estado líquido

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ABSTRACT

In this work, mouse epididymal sperm was kept in a liquid medium to compare pentoxifylline and trehalose. Twenty adult male mice were used, and epididymal spermatozoa were divided into four groups: control, pentoxifylline 1 mM, pentoxifylline 3 mM, and trehalose 50 mM. A modified Tris-based extender was used as the basal storage medium. Motility and chlortetracycline based capacitation findings were recorded at 0, 24 and 48 hours. Total antioxidant status, total oxidant status and oxidative stress index measurements were performed at 0 and 48 hours. Motility decreased in all groups during storage. A significant difference among the groups was observed only at 0 hours, with the highest value in the trehalose 50 mM group. At the initial time point, total antioxidant status values were close to each other. After 48 hours, higher total antioxidant status values were obtained in the pentoxifylline 3 mM and trehalose 50 mM groups compared with the control. The lower oxidative stress index values observed at 48 hours in the pentoxifylline 3 mM and trehalose 50 mM groups appear to be mainly related to differences in total antioxidant status levels, as total oxidant status values did not differ significantly among treatments. According to the chlortetracycline results, the groups were similar at 0 hours. At 24 hours, capacitated sperm percentages were higher in all treated groups, while the highest value at 48 hours was observed in the trehalose 50 mM group. These findings suggest that short-term storage influenced redox balance and capacitation-related membrane changes more clearly than the preservation of motility. Among the tested additives, trehalose 50 mM showed the most consistent protective profile.

Key words: Mouse epididymal sperm; liquid storage; pentoxifylline; trehalose; oxidative stress.

RESUMEN

En este estudio se compararon los efectos de la pentoxifilina y la trehalosa sobre espermatozoides epididimarios de ratón durante el almacenamiento líquido. Se utilizaron veinte ratones machos adultos, y los espermatozoides epididimarios se distribuyeron en cuatro grupos: control, pentoxifilina 1 mM, pentoxifilina 3 mM y trehalosa 50 mM. Como medio basal se empleó un diluyente modificado a base de Tris. La motilidad y la capacitación evaluada mediante tinción con clortetraciclina se registraron a las 0, 24 y 48 horas; asimismo, el estado antioxidante total, el estado oxidante total y el índice de estrés oxidativo se determinaron a las 0 y 48 horas. Durante el almacenamiento, la motilidad disminuyó en todos los grupos. Sin embargo, solo se detectó una diferencia significativa a las 0 horas, con el valor más alto en el grupo trehalosa 50 mM. Inicialmente, los valores de estado antioxidante total fueron similares. Después de 48 h, la pentoxifilina 3 mM y la trehalosa 50 mM mostraron valores de estado antioxidante total más altos que el control. En estos mismos grupos, los menores valores de índice de estrés oxidativo observados a las 48 horas parecen estar relacionados principalmente con diferencias en los niveles de estado antioxidante total, dado que los valores de estado oxidante total no difirieron significativamente entre los tratamientos. Según los resultados de clortetraciclina, los grupos fueron similares a las 0 horas. A las 24 horas, los porcentajes de espermatozoides capacitados fueron mayores en todos los grupos tratados, mientras que a las 48 horas el valor más alto se observó con trehalosa 50 mM. En conjunto, estos hallazgos sugieren que el almacenamiento líquido a corto plazo influyó más claramente sobre el equilibrio redox y los cambios de membrana relacionados con la capacitación que sobre la conservación de la motilidad. Entre los aditivos evaluados, la trehalosa 50 mM mostró el perfil protector más consistente.

Palabras clave: Espermatozoides epididimarios de ratón; almacenamiento líquido; pentoxifilina; trehalosa; estrés oxidativo.

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INTRODUCTION

The preservation and transfer of valuable mouse lines is still important in biomedical research. In many laboratories, live animals are still sent for this purpose. However, this method is not always easy or suitable. It can cause high transportation cost, some logistical problems, animal welfare concerns and also possible biosafety risks. Because of these reasons, alternative methods for transferring genetic material between research centers have become more important. In this regard, short-term storage of epididymal sperm at refrigerated temperature is considered as a simple and useful method for the transport of mouse lines [1].

The idea of using refrigerated mouse epididymal sperm has been investigated for some time. Early studies reported that sperm obtained from epididymides kept under cold conditions could maintain progressive motility for several days, indicating that short-term transport may be technically feasible [2]. Later, this approach was further supported by studies showing that cold-stored cauda epididymides could be used successfully for in vitro fertilization (IVF) and embryo transfer. These findings made the method particularly useful for the exchange of genetically engineered mouse (Mus musculus) lines between laboratories [3].

Despite these advantages, sperm quality decreases during storage. Motility, membrane integrity and fertilizing ability are negatively affected with time. This situation limits the practical storage period. Previous studies using additives such as dimethyl sulfoxide and quercetin showed that the composition of the storage medium has an important effect on sperm survival, motility and fertility at 4 °C [4]. In addition, sperm collected from cold-stored epididymides may still have fertilizing capacity after cryopreservation and thawing. This also shows the importance of improving this preservation step [5].

The decrease in sperm quality during cold storage is closely related to membrane damage and oxidative stress. The sperm plasma membrane is very sensitive to cooling because temperature changes can affect its lipid structure. This is especially important in mature spermatozoa, because their membranes contain high amounts of polyunsaturated fatty acids. For this reason, they are more sensitive to peroxidative damage [6, 7].

At the same time, sperm cells have limited antioxidant capacity. During storage, reactive oxygen species may increase and cause oxidative stress in the cellular environment. This situation can damage membrane stability, motility, protein function, DNA integrity and fertilizing ability. Therefore, the evaluation of oxidant and antioxidant balance is important in sperm preservation studies [8, 9].

Total antioxidant status (TAS) and total oxidant status (TOS) are commonly used to evaluate the general oxidant-antioxidant status of biological samples. When TAS and TOS are evaluated together with oxidative stress index (OSI), they may give more information about storage-related oxidative damage [10,11].

Recent studies showed that OSI may be useful for predicting semen cryotolerance. This also supports the importance of OSI in preservation studies [12]. However, biochemical markers alone may not be enough to evaluate sperm function. Functional membrane changes, especially capacitation-related changes, should also be evaluated. Chlortetracycline (CTC) staining is one of the classical methods used to determine capacitation-related changes in spermatozoa [13].

Among the additives used in sperm preservation, pentoxifylline and trehalose are important because they may affect sperm cells by different mechanisms. Pentoxifylline is mainly known as a motility-stimulating agent. It has been used in assisted reproduction to activate immotile epididymal or testicular spermatozoa [14,15].

In addition to its effect on motility, pentoxifylline has also been reported to support capacitation and acrosome reaction without decreasing viability in cryopreserved horse (Equus caballus) epididymal sperm. This shows that pentoxifylline may also affect functional membrane-related events [16]. Trehalose is a non-reducing disaccharide and is known for its membrane-protective effect under cold and cryogenic conditions. However, its protective effect may change according to species, concentration and preservation protocol [17,18].

Studies on goat (*Capra hircus*) sperm also showed that trehalose and pentoxifylline may reduce damage caused by chilling and freezing. These effects may occur when they are used alone or together [19].

Although the cold storage of mouse epididymal sperm has practical importance, there are still limited controlled studies evaluating how different additives influence both sperm function and oxidative balance during short-term liquid storage. Therefore, the present study was planned to investigate the effects of pentoxifylline and trehalose on mouse epididymal sperm stored at +4 °C. For this aim, spermatological parameters, capacitation status and redox-related markers such as TAS, TOS and OSI were examined. It was thought that these additives may help to maintain sperm quality during short-term storage because of their possible effects on motility and membrane protection.

MATERIALS AND METHODS

Animals, chemicals, and ethical approval

Pentoxifylline (P1784; Sigma-Aldrich, St. Louis, MO, USA) and D(+)-trehalose dihydrate (T0167; Sigma-Aldrich, St. Louis, MO, USA) were used in this study as additives in the sperm storage medium. Other chemicals were used as analytical grade, unless a different grade was indicated.

The study material consisted of 20 adult male mice, aged 10–12 weeks, weighing approximately 30 ± 5 g. Body weight was measured using an electronic balance (PCB 1000-2, Kern & Sohn GmbH, Germany). The animals were kept in the Experimental Animals Application and Research Center of Aksaray University. Commercial pellet feed and water were provided *ad libitum*. The room temperature was kept around 23 ± 2 °C, and a 12 hours (h) light and 12 h dark cycle was used. The animals were checked two times a day for their general health condition and vitality.

The study protocol, entitled “Evaluation of Mouse Epididymal Sperm by Liquid Storage at +4 °C”, was approved by the Aksaray University Local Ethics Committee for Animal Experiments on 24

April 2025 (meeting no. 2025/04, decision no. 26). The approval covered the use of 20 mice.

Experimental design and preparation of the storage medium

The animals were anesthetized intraperitoneally with Ketamine and Xylazine at doses of 90 mg/kg and 10 mg/kg, respectively. Following anesthesia, the reproductive organs were carefully exteriorized, surrounding adipose tissues were removed, and the epididymides were isolated.

A modified Tris based stock extender was prepared as the basal storage medium, based on the extender formulation previously reported by Bucak *et al*. [20]. The extender contained 297.58 mM Tris, 96.32 mM citric acid, 82.66 mM fructose, 100 IU/mL Penicillin G, and 1 mg/mL Streptomycin. This basal medium was used to establish four experimental groups:

Group 1 (Control, n = 5): epididymal spermatozoa stored in basal extender only.

Group 2 (Pentoxifylline 1 mM, n = 5): epididymal spermatozoa stored in basal extender supplemented with 1 mM pentoxifylline.

Group 3 (Pentoxifylline 3 mM, n = 5): epididymal spermatozoa stored in basal extender supplemented with 3 mM pentoxifylline.

Group 4 (Trehalose 50 mM, n = 5): epididymal spermatozoa stored in basal extender supplemented with 50 mM trehalose.

Recovery and liquid storage of epididymal spermatozoa

The left cauda epididymis was washed gently with phosphate buffered saline (PBS; pH approximately 7.4) and then transferred into the prepared storage medium. To release spermatozoa, several small incisions were made in the epididymal tissue. The tissue was kept in a Petri dish at 37 °C for 30 min. At the end of this period, spermatozoa were dispersed into the medium. The samples were then kept at +4 °C (KGN55CWE0N, Bosch, Germany) until the analyses were performed.

Spermatological parameters were assessed at 0, 24, and 48 h of storage. Biochemical analyses were performed at 0 and 48 h.

Spermatological evaluation

Sperm concentration was evaluated after diluting the sperm suspension at a ratio of 1:100. The diluted sample was loaded into a Neubauer hemocytometer, and the counted spermatozoa were used to calculate the sperm concentration of each sample.

Sperm motility was examined using a phase contrast microscope equipped with a heated stage (CX41/CX41RF, Olympus Corporation, Tokyo, Japan). The evaluations were performed under ×40 and ×100 objective magnifications when required. Motility results were recorded as the percentage of motile spermatozoa.

Sperm capacitation status was examined by chlortetracycline fluorescence staining. The method was applied with small modifications from previously reported mouse CTC staining protocols [21]. For staining, 5 µL sperm suspension was mixed with 20 µL freshly prepared CTC working solution. This solution included 750 µM CTC-HCl, 130 mM NaCl, 20 mM Tris-HCl and 5 mM D,L-cysteine, and the pH was adjusted to 7.8. All staining steps were performed under low light conditions.

The staining reaction was stopped by adding 1 M Tris-HCl solution containing 1 % (v/v) glutaraldehyde at pH 7.4. Then, a small volume of the stained sample was placed on a microscope slide and covered with a coverslip. The slides were examined under a fluorescence microscope (Axioscope 5, Carl Zeiss, Jena, Germany) equipped with an Axiocam 208 color camera [21].

A total of 200 spermatozoa were examined for each sample. The evaluation was performed according to mouse-adapted CTC fluorescence patterns. Spermatozoa with uniform bright fluorescence over the whole head were accepted as non-capacitated spermatozoa (F pattern). Spermatozoa showing bright fluorescence in the anterior head region together with a dark band in the equatorial or post-acrosomal region were accepted as capacitated spermatozoa (B pattern) [22].

Biochemical analyses

The oxidative status of the samples was assessed by measuring TAS and TOS. TAS was analyzed with the Elabscience TAS Colorimetric Assay Kit (Cat. No. E-BC-K801-M). TOS was analyzed with the Elabscience TOS Colorimetric Assay Kit (Cat. No. E-BC-K802-M). Absorbance values were measured using a Humalyzer 3000 semi-automatic photometer (Diagnostics, Wiesbaden, Germany). The OSI was then calculated from TAS and TOS values after the required unit conversion. This value was used to obtain a general estimate of the oxidant-antioxidant balance.

Statistical analysis

All data were expressed as mean ± standard deviation. The normality of the data was assessed before statistical comparisons. Since the groups were compared separately at each storage time, one-way analysis of variance was used for 0, 24 and 48 h results. When a significant difference was found, Tukey's post hoc test was used for comparison between groups.

In addition to significance testing, effect size analysis was performed to estimate the magnitude of the observed differences. Eta squared (η^2) and omega squared (ω^2) were calculated for one way ANOVA, and Hedges' g was used for pairwise comparisons. The value of $P < 0.05$ was accepted as statistically significant. Statistical analyses were performed using IBM SPSS Statistics version 27.

RESULTS AND DISCUSSION

Total antioxidant status values of the experimental groups are presented in TABLE I. At 0 h, no statistically significant difference was observed among the groups (P = 0.0569), indicating that baseline TAS values were broadly comparable. At 48 h, however, the overall group effect became significant (P = 0.0112). The lowest TAS value was recorded in the control group, whereas the Pen 3 mM and Trehalose 50 mM groups showed significantly higher values. The Pen 1 mM group displayed intermediate values and did not differ significantly from either the control group or the higher TAS groups.

TABLE I

Total antioxidant status (µmol/L) values of mouse epididymal sperm samples stored at +4 °C in the experimental groups

Groups	0 h	48 h
Control	3665.46 ± 369.96	3212.10 ± 391.03[b]
Pen 1 mM	4430.67 ± 545.28	3648.86 ± 463.53[ab]
Pen 3 mM	4360.83 ± 578.32	4068.66 ± 107.71[a]
Trehalose 50 mM	4582.08 ± 550.48	4074.12 ± 527.29[a]
P value	0.0569	0.0112

Values are presented as mean ± SD (n = 5). TAS values are expressed as µmol/L. Control: non-supplemented group; Pen 1 mM: group supplemented with 1 mM pentoxifylline; Pen 3 mM: group supplemented with 3 mM pentoxifylline; Trehalose 50 mM: group supplemented with 50 mM trehalose. TAS: total antioxidant status; h: hour; SD: standard deviation; mM: millimolar. Different superscript letters in the same column show statistically significant differences among groups by one-way ANOVA followed by Tukey post hoc test (P < 0.05). There was no significant difference among groups at 0 h, whereas a significant difference was observed at 48 h.

Total oxidant status values are summarized in TABLE II. No significant differences were detected among the groups either at 0 h (P = 0.6361) or at 48 h (P = 0.5539). Although the control group showed numerically higher TOS values at 48 h than the treated groups, these differences were not statistically significant.

TABLE II

Total oxidant status (µmol/L) values of mouse epididymal sperm samples stored at +4 °C in the experimental groups

Groups	0 h	48 h
Control	14.05 ± 1.79	20.41 ± 4.07
Pen 1 mM	13.23 ± 3.55	17.28 ± 4.15
Pen 3 mM	13.59 ± 3.58	17.67 ± 4.87
Trehalose 50 mM	11.87 ± 1.28	17.25 ± 2.49
P value	0.6361	0.5539

Values are presented as mean ± SD (n = 5). TOS values are expressed as µmol/L. Control: non-supplemented group; Pen 1 mM: group supplemented with 1 mM pentoxifylline; Pen 3 mM: group supplemented with 3 mM pentoxifylline; Trehalose 50 mM: group supplemented with 50 mM trehalose. TOS: total oxidant status; h: hour; SD: standard deviation; mM: millimolar. No significant difference was observed among groups at 0 h or 48 h according to one-way ANOVA (P > 0.05).

The OSI results are shown in TABLE III. At 0 h, the overall group comparison did not reach statistical significance (P = 0.0671). By 48 h, a significant difference among groups was observed (P = 0.0266). The control group had the highest OSI value, whereas lower values were found in the Pen 3 mM and Trehalose 50 mM groups. The Pen 1 mM group showed an intermediate profile and did not differ significantly from either the control or the lower OSI groups.

TABLE III

Oxidative stress index values of mouse epididymal sperm samples stored at +4 °C in the experimental groups

Groups	0 h	48 h
Control	0.39 ± 0.10	0.64 ± 0.14[a]
Pen 1 mM	0.30 ± 0.08	0.47 ± 0.10[ab]
Pen 3 mM	0.31 ± 0.08	0.44 ± 0.12[b]
Trehalose 50 mM	0.26 ± 0.01	0.43 ± 0.08[b]
P value	0.0671	0.0266

Values are presented as mean ± SD (n = 5). OSI values were calculated by using TAS and TOS results. Control: non-supplemented group; Pen 1 mM: group supplemented with 1 mM pentoxifylline; Pen 3 mM: group supplemented with 3 mM pentoxifylline; Trehalose 50 mM: group supplemented with 50 mM trehalose. OSI: oxidative stress index; TAS: total antioxidant status; TOS: total oxidant status; h: hour; SD: standard deviation; mM: millimolar. Different superscript letters in the same column show statistically significant differences among groups according to one-way ANOVA followed by Tukey post hoc test (P < 0.05). No significant difference was found among groups at 0 h, so superscript letters were not used in this column.

The more balanced profile observed in the trehalose 50 mM group suggests that the additives primarily supported functional integrity rather than simply preserving movement. In liquid cold storage, the effects of antioxidant supplementation are strongly dependent on species, extender composition, and dose, meaning that a protective effect observed in one model may not necessarily be reproduced in another [23]. Increased oxidative burden may compromise sperm function through lipid peroxidation, protein oxidation, and DNA damage [9].

The fact that structural and biochemical improvement did not translate into sustained motility in the present study suggests that the storage related injury was not only mechanical but also strongly redox dependent [24]. At the same time, sperm physiology does not require suppression of all oxidative activity; rather, oxidative events must be maintained within a physiologically functional range [25]. Therefore, the divergence observed here between unchanged TOS and altered TAS/OSI is best interpreted as evidence of improved antioxidant buffering rather than a true reduction in total oxidant load [26].

Motility values of the groups at 0, 24, and 48 h are presented in TABLE IV. A significant difference among groups was observed at 0 h (P = 0.0055), with the highest motility value recorded in the Trehalose 50 mM group. In contrast, no statistically significant group differences were detected at 24 h (P = 0.1571) or 48 h (P = 0.4121). Regardless of treatment, motility declined over time in all groups during storage.

TABLE IV

Motility (%) values of mouse epididymal sperm samples stored at +4 °C in the experimental groups

Groups	0 h	24 h	48 h
Control	48.00 ± 7.58^{b}	39.00 ± 7.42	15.00 ± 3.54
Pen 1 mM	55.00 ± 6.12^{ab}	40.00 ± 6.12	18.00 ± 4.47
Pen 3 mM	53.00 ± 2.74^{b}	42.00 ± 5.70	17.00 ± 2.74
Trehalose 50 mM	64.00 ± 6.52^{a}	49.00 ± 8.94	19.00 ± 2.24
P value	0.0055	0.1571	0.4121

Values are presented as mean ± SD (n = 5). Motility values show the percentage of motile spermatozoa. Control: sperm samples without supplementation; Pen 1 mM: sperm samples with 1 mM pentoxifylline; Pen 3 mM: sperm samples with 3 mM pentoxifylline; Trehalose 50 mM: sperm samples with 50 mM trehalose. h: hour; SD: standard deviation. Different superscript letters in the same column indicate statistically significant differences among groups by one-way ANOVA followed by Tukey's post hoc test (P < 0.05). No significant difference was found among groups at 24 h or 48 h.

In the present study, the effects of the tested additives were more apparent in redox balance and membrane responsiveness assessed by CTC than in motility. For this reason, cold transport of epididymides or epididymal sperm has been developed as a practical and biologically safer alternative to live animal shipment (Takeo *et al*. [1]). Likewise, the ability of dimethyl sulfoxide and quercetin to prolong survival, motility, and fertility in cold stored mouse sperm has highlighted the importance of medium composition in determining storage success [4]. In this context, current approaches to mouse sperm evaluation increasingly emphasize that motility alone is insufficient and that membrane function, acrosomal status, and other functional markers should also be considered [27].

The percentage of capacitated spermatozoa determined by CTC staining is shown in TABLE V. At 0 h, the difference among groups was not statistically significant (P = 0.0536). At 24 h, a significant group effect was observed (P = 0.0002), with all treated groups showing higher CTC percentages than the control group. At 48 h, the difference among groups remained significant (P = 0.0001). At this time point, the Trehalose 50 mM group had the highest CTC percentage and differed significantly from the other groups, while the control, Pen 1 mM, and Pen 3 mM groups did not differ significantly from each other.

TABLE V

Capacitated sperm percentage determined by chlortetracycline staining (%) in mouse epididymal sperm samples stored at +4 °C in the experimental groups

Groups	0 h	24 h	48 h
Control	36.46 ± 1.86	38.42 ± 1.51^{b}	32.03 ± 0.80^{b}
Pen 1 mM	36.97 ± 3.87	40.85 ± 0.45^{a}	33.38 ± 0.73^{b}
Pen 3 mM	38.04 ± 1.17	40.91 ± 1.26^{a}	32.43 ± 0.97^{b}
Trehalose 50 mM	40.93 ± 2.34	42.55 ± 0.65^{a}	35.88 ± 1.48^{a}
P value	0.0536	0.0002	0.0001

Values are presented as mean ± SD (n = 5). CTC values show the percentage of capacitated spermatozoa. Control: non-supplemented group; Pen 1 mM: group supplemented with 1 mM pentoxifylline; Pen 3 mM: group supplemented with 3 mM pentoxifylline; Trehalose 50 mM: group supplemented with 50 mM trehalose. CTC: chlortetracycline staining; h: hour; SD: standard deviation; mM: millimolar. Different superscript letters in the same column show statistically significant differences among groups according to one-way ANOVA followed by Tukey post hoc test (P < 0.05). No significant difference was found among groups at 0 h, but significant differences were found at 24 h and 48 h.

The results obtained with trehalose are mechanistically consistent. Because trehalose does not readily penetrate the cell, it is thought to exert its effects mainly at the extracellular membrane interface, where it can buffer osmotic stress and stabilize lipid organization during cooling [18].

In goat sperm, trehalose has been shown to improve membrane fluidity, which may help explain the better membrane response observed in the present study through CTC staining [28].

In bovine (*Bos taurus*) sperm, trehalose supplementation has also been associated with improved oxidative stress variables, indicating that its effects may extend beyond structural stabilization to redox regulation [29].

Consistent with these findings, studies on human sperm have also shown that trehalose may improve motility, membrane integrity, and mitochondrial function. This supports the interpretation that the more favorable redox balance and CTC profile observed in the trehalose 50 mM group at 48 h in the present study may be related to its membrane-stabilizing and cell-protective effects [17].

Similarly, the finding that different sugars can alter motility, morphology, and DNA damage during liquid storage of rat epididymal sperm at 4 °C indicates that both osmotic regulation and energy support may strongly affect storage outcome [30]. The high susceptibility of the sperm plasma membrane to cooling, due to its cholesterol content and abundance of polyunsaturated fatty acids, also helps explain why trehalose may have acted mainly at the level of membrane stabilization in the present model [7].

The pattern observed with pentoxifylline was narrower, but still biologically meaningful. Given that mammalian capacitation is highly dependent on cAMP/PKA signaling and ion fluxes, the early effect of pentoxifylline on CTC patterns was biologically expected [31].

At the same time, recent structure based studies suggest that the functional window between beneficial phosphodiesterase modulation and excessive stimulation may be relatively narrow for pentoxifylline like compounds [32]. Although pentoxifylline has been useful in initiating motility in immotile epididymal and testicular sperm for ICSI, these effects have generally been interpreted as short term functional gains rather than as evidence of broad preservation [14].

Thus, in the present study, the increase in CTC values at 24 h in the absence of a sustained motility advantage suggests that pentoxifylline preserved signaling competence more effectively than long term movement. A major strength of CTC staining is its ability to distinguish fluorescence patterns associated with capacitation and acrosomal status [13]. However, capacitation like changes induced by cold or cryostress do not always correspond directly to true fertilizing competence [33].

CONCLUSIONS AND IMPLICATIONS

Under the present experimental conditions, short term liquid storage of mouse epididymal sperm at +4 °C was influenced more clearly at the level of redox balance and capacitation associated membrane status than at the level of sustained motility.

Among the tested additives, trehalose at 50 mM showed the most consistent protective effect, whereas pentoxifylline at 3 mM provided a more limited but still meaningful benefit. These findings suggest that improvement during chilled storage should not be judged solely by motility, since membrane related functional integrity and oxidative balance appeared to be more sensitive indicators of preservation success.

Overall, trehalose at 50 mM may be considered a promising additive for short term liquid storage of mouse epididymal sperm, although its practical value should be confirmed in future studies including fertilization outcomes.

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Conflicts of interest

The authors declare no conflict of interest.

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