Submitted: 30 September, 2023 Accepted: 29 December, 2023 Published: 30 June, 2024 DOI:10.22514/j.androl.2024.012

REV INT ANDROL
Revista Internacional de Andrología

ORIGINAL RESEARCH

Static magnetic field can ameliorate detrimental effects of cryopreservation on human spermatozoa

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Abstract

This study aims to improve the freezing-thawing process of human sperm using a static magnetic field. The study included 25 normozoospermic human samples. After an initial evaluation of sperm parameters, samples were prepared by the direct swim-up method. Before freezing, sperm motility, viability, morphology, acrosome reaction and DNA fragmentation rate were assessed. The samples were divided into 4 groups: 0, 1, 5 and 10 mT, and each group was frozen by the rapid freezing method. After thawing, the parameters were re-evaluated and compared between groups. Sperm motility decreased significantly during cryopreservation in all groups. The static magnetic field did not protect against decreased progressive motility after freezing, but the total sperm motility was significantly higher in the 10 mT group compared to the other groups. Sperm viability was higher in the 10 mT group than in the other groups. There was no significant difference in the rate of normal sperm morphology after freezing. The rate of spermatozoa with intact acrosome decreased after freeze-thawing, and the static magnetic field did not protect against the acrosome reaction. The rate of DNA integrity was significantly higher in the 10 mT group compared to the other groups. A static magnetic field with an intensity of 10 mT improved sperm viability and DNA integrity compared to other groups. However, it did not provide significant protection against decreased sperm motility or acrosome reaction.

Keywords

Rapid freezing; Sperm cells; Cryoinjury; DNA integrity; Acrosome reaction

Campo magnético estático puede mejorar los efectos perjudiciales de la criopreservación en espermatozoides humanos

Resumen

Este estudio tiene como objetivo mejorar el proceso de congelación-descongelación de los espermatozoides humanos mediante un campo magnético estático. El estudio incluyó 25 muestras humanas normozoospérmicas. Tras una evaluación inicial de los parámetros espermáticos, las muestras se prepararon mediante el método de natación directa. Antes de la congelación, se evaluaron la motilidad, la viabilidad, la morfología, la reacción acrosómica y la tasa de fragmentación del DNA de los espermatozoides. Las muestras se dividieron en 4 grupos: 0, 1, 5 y 10 mT, y cada grupo se congeló mediante el método de congelación rápida. Tras la descongelación, los parámetros se reevaluaron y se compararon entre los grupos. La motilidad de los espermatozoides disminuyó significativamente durante la criopreservación en todos los grupos. El campo magnético estático no protegió contra la disminución progresiva de la motilidad después de la congelación, pero la motilidad total de los espermatozoides fue significativamente mayor en el grupo de 10 mT en comparación con los demás grupos. La viabilidad de los espermatozoides fue mayor en el grupo de 10 mT que en los demás grupos. No hubo diferencias significativas en la tasa de morfología normal de los espermatozoides después de la congelación. La tasa de espermatozoides con acrosoma intacto disminuyó después de la congelación-descongelación, y el campo magnético estático no protegió contra la reacción acrosómica. La tasa de integridad del DNA fue significativamente mayor en el grupo de 10 mT en comparación con los otros grupos. Un campo magnético estático con una intensidad de 10 mT mejoró la viabilidad de los espermatozoides y la integridad del DNA en comparación con otros grupos. Sin embargo, no proporcionó una protección significativa contra la disminución de la motilidad de los espermatozoides o la reacción acrosómica.

Palabras Clave

Congelación rápida; Espermatozoides; Criolesión; Integridad del DNA; Reacción acrosómica

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1. Introduction

Cryobiology is a science of preserving cells or tissues for a long period at a very low temperature. Sperm cryopreservation is widely used in *in vitro* fertilization clinics worldwide. Sperm cryopreservation is mainly indicated for testicular sperm extraction in azoospermia, before cancer treatment, vasectomy, gender reassignment, hypogonadotropic hypogonadism and spinal cord injury [1–3]. Cryopreservation may have detrimental effects on sperm membrane integrity, motility, viability and DNA integrity [2, 4]. Additionally, sperm freezing can lead to a loss of sperm surface proteins, which are essential for fertilization. During different stages of freezing, the inactivation of membrane-bound enzymes and alteration in the distribution of proteins within the membrane are observed [4].

Magnetic fields have been studied in various fields such as proliferation and differentiation, transplantation, cell death, immune system, as well as freezing [5]. According to previous studies, the plasma membrane of cells is affected by magnetic fields [6]. The magnetic field affects the function of membrane-gated channels, especially calcium ion channels, and causes an increase in intracellular calcium [7]. The magnetic field affects the rotation of membrane phospholipids and changes the distribution of membrane proteins, causing a change in the arrangement of the plasma membrane, which is caused by the diamagnetism of the plasma membrane components [8]. Magnetic fields are static- and variable magnetic field. In contrast to variable magnetic fields, a static magnetic field (SMF) is constant and does not alter in intensity or direction over time. Most studies indicate that SMF increases the permeability of the membrane by reducing its fluidity and increasing its hardness, leading to greater biophysical stability of the membrane [9, 10].

Using SMF to reduce cryoinjuries has gained popularity in recent years. It has been demonstrated that a magnetic field with an intensity of 0.4 and 0.8 Tesla can increase the survival of blood cells after the freeze-thaw process by enhancing the biophysical stability of the membrane [10]. Tablado *et al.* [11] conducted a study to investigate the effect of the magnetic field on the morphology and morphometry of mouse epididymal sperm, using a SMF with an intensity of 0.7 Tesla. The results of the study showed that the magnetic field did not have a harmful effect on the spermatogenesis of mice [11].

SMF can also modify the orientation of spermatozoa. Emura *et al.* [12] showed that cow sperm can be found in a vertical orientation with a magnetic field with a maximum intensity of 1.7 Tesla, caused by the components of the plasma membrane (lipid bilayer membrane and transmembrane proteins), DNA in the head of the sperm, and microtubules in the tail of the sperm. The effect of SMF (1, 5, 10 millitesla, mT) on the sperm motility of Danube Hochen fish and trout showed that the magnetic field increases the speed of the sperm, resulting in an increased chance of conception and fertility [13, 14]. Regarding the effect of SMF on ovarian vitrification, the use of SMF with an intensity of 1 mT was shown to reduce damage caused by tissue ischemia and increase the release of fibroblast growth factor-2 (FGF-2) from endothelial cells, causing angiogenesis, establishment of blood flow and delay of cell death. Also, more primordial follicles were preserved in ovaries exposed to an SMF before transplantation [15]. Additionally, it was shown that exposing the ovary to an SMF during the freezing process leads to better protection of the follicles against damage caused by freezing and increases their resistance to cryoinjuries [16, 17].

The current research was conducted with the aim of improving the freezing-thawing process of human sperm using a SMF. The use of this magnetic field has fewer variable parameters and creates milder effects on biological systems, which is beneficial for humans in particular. Additionally, a review of the literature shows that SMF can have a positive effect on the optimization of sperm freezing in animal studies. However, searches conducted to date show that no study has been conducted on the use of SMF for freezing human sperm.

2. Materials and methods

2.1 Sample preparation

Twenty-five normal semen samples according to World Health Organization (WHO) guidelines [18], from men who referred to Gandhi *in vitro* fertilization (IVF) clinic for infertility workup were included in this prospective study. The males' age range was 25–39 years. Men with a history of varicocele and heavy smoking were excluded from the study. After 3–4 days of abstinence, the specimens were collected by masturbation into specific sterile containers. Semen analysis was performed following WHO guidelines [18]. Direct swim-up was used for sperm preparation.

2.2 Study design

After sperm preparation, the samples were divided into four groups; 0 mT: freezing the samples without exposure to SMF, 1 mT: samples were exposed to 1 mT SMF during the equilibration step of freezing, 5 mT: samples were exposed to 5 mT SMF during the equilibration step of freezing, 10 mT: samples were exposed to 10 mT SMF during the equilibration step of freezing.

2.3 Static magnetic field production

In this study, the electromagnetic device designed by the Biophysics Department of Tarbiat Modares University was used. The device was capable of producing a magnetic field of 0.5 to 30 mT. The magnetic field was generated using two poles wrapped with 180 turns of copper wire with a diameter of 2.5 mm. This setup generated a SMF in the space between the poles and was powered by an electric energy supply source (0–50 V with a maximum power of 1 kW). A tesla meter (PHYWE Gottingen 13610.93, Gottingen, Germany) was used to adjust the device and measure the uniformity of the magnetic field [19].

2.4 Freeze-thaw process

After the sperm preparation, the sperm samples were divided into four different groups, as mentioned above and placed in separate cryovials. The sperm sample and freezing medium (Sage, USA) were mixed slowly in a 1:1 ratio and exposed to different intensities of SMF during the equilibrium stage

at room temperature (3 minutes). Following this stage, the samples were placed in a refrigerator at 4 °C for 30 minutes. Finally, the samples were immediately placed in nitrogen vapor for 30 minutes and then immersed in liquid nitrogen (−196 °C) for storage for at least 2 weeks. For thawing, the cryovials were first brought to room temperature and then placed in 35 °C water. Ham's F-10 medium containing human serum albumin was slowly added to the cryovials. After a 5-minute centrifuge at 2000 rpm, the supernatant was removed and the remaining sample was assessed for further evaluation.

2.5 Evaluation of sperm motility, viability and morphology

To check sperm motility, at least 200 spermatozoa were assessed for progressive motility, non-progressive motility, and immobility across 5 fields of view according to WHO guidelines [18]. The viability of sperm was evaluated using the eosin-nigrosin method: spermatozoa with a red or dark pink head were considered dead, while those with a white or pale pink head were deemed alive. Results were reported as a percentage, based on assessment of at least 200 spermatozoa [18]. Papanicolaou staining was utilized to evaluate sperm morphology in line with WHO guidelines, using 10 microliters of the sample to create a spread (smear) on each slide. The resulting slides were fixed and stained simultaneously. Abnormalities in head, midpiece and tale of sperm cells were evaluated and the rate of normal morphology was reported as percentage.

2.6 Evaluation of the acrosome reaction

First, an equal ratio of a sperm sample was mixed with 3% glutaraldehyde and fixed in the dark. After simple centrifugation for 5 minutes, half of the supernatant was removed and replaced with distilled water. Then, a smear was prepared from the sample and dried at room temperature. Smears were stained with 8% Bismarck Brown dye for 10 minutes and washed. The second staining was done using 8% Rose Bengal for 20 minutes. After washing and drying, slides were examined using a light microscope with a magnification of 1000, and at least 200 spermatozoa were examined. Spermatozoa whose acrosome area was seen in bright red color were considered to have performed acrosome reaction, and spermatozoa whose acrosome area was colorless were considered not to have performed acrosome reaction [20].

2.7 Evaluation of DNA integrity

This test was performed according to the instructions provided in the kit purchased from Ideh Varzan Farda Company (SD 062202, Tehran, Iran). First, 70 μL of agarose gel was mixed with 30 μL of 15–20 million sperm suspension. 50 μL of the sample was, placed on a slide and covered with a coverslip, which was placed in the refrigerator for 5 minutes. Then the coverslip was removed and placed in the denaturing solution containing hydrocholoride (HCL) acid for 7 minutes. After that, it was placed in mercaptoethanol lysing solution for 15 minutes. The spermatozoa were washed and de-watered. Then, staining was done with Wright and the slide was evaluated with a light microscope at 1000 magnification. Spermatozoa with a large or medium halo were considered normal and the cells with a small or no halo were considered abnormal. At least 200 spermatozoa were examined, and the percentage of normal and abnormal spermatozoa were reported [21].

2.8 Statistical analysis

Data distribution was assessed using the Shapiro-Wilk Test. To compare means between different groups, we utilized the data distribution of each group. One-way analysis of variance with Tukey's *post hoc* test was used for groups with normal distribution, while Kruskal-Wallis test with Dunnett's *post hoc* test was used for groups with non-normal distribution. The statistical significance was $p < 0.05$, and the hypotheses were considered as one-tailed.

3. Results

Table 1 presents the sperm parameters included in this study. During the freezing process, the sperm motility significantly decreased in the 0, 1 and 5 mT groups compared to before freezing. During the freezing process, the rate of progressive motility decreased significantly in different groups. SMF did not have a protective effect on progressive motility in any of the groups, even though this parameter increased with the increase in the intensity of the magnetic field, but it did not create a significant difference. Total motility after freezing in the SMF group with an intensity of 10 mT was not significantly different compared to the group before freezing.

The sperm viability rate before freezing was 89.57 ± 9.78, which significantly decreased after the freeze-thaw process. Treatment with an SMF with a dose of 10 mT led to an improvement in sperm viability compared to the 0 mT, 1 mT and 5 mT. Also, there was no significant difference between the mean viability in the 10 mT and before freezing group.

The rate of sperm normal morphology after freezing showed a significant decrease. Although an increase in the normal morphology of sperm was seen with the increase in the intensity of the magnetic field, it could not create a significant difference.

The rate of spermatozoa that did not perform acrosome reaction before freezing was 72.27 ± 12, which decreased after the freezing-thawing process and reached 56.91 ± 13.06 in the 0 mT group. Our results showed that the SMF could not have a protective effect on the acrosome reaction of spermatozoa (Table 2).

Spermatozoa with intact DNA during the freezing-thawing process significantly decreased in the 0, 1 and 5 mT groups, but the 10 mT group did not show a significant difference with the group before freezing. Also, the rate of normal DNA in the 10 mT group was higher compared to the 0 and 1 mT groups (Fig. 1). The results of eosin-nigrosin, Papanicolaou, double staining and SCD are shown in Fig. 2.

4. Discussion

It appears that the SMF can affect cellular systems, such as the sensitivity of biomolecules, intracellular structural changes and enzyme reactions [5]. Baniasadi and colleagues recently

TABLE 1. Sperm parameters of semen included in this study.

Parameters	Mean	Minimum	Maximum	Standard deviation
Age (yr)	37.10	25	39	2.13
Sperm count (10^6/mL^{-1})	110.50	40	210	15.26
Progressive motility (%)	62.34	32	72	12.40
Non-progressive motility (%)	7.52	2	21	5.14
Total motility (%)	69.26	48	89	10.81
Immotile (%)	30.74	10	45	10.71
Normal morphology (%)	5.65	4	15	1.35
Round cells (10^6/mL^{-1})	465.00	100	1000	153.50

TABLE 2. Comparison of sperm parameters among different experimental groups after treatment with static magnetic field.

Parameters (%)	Before freezing	0 mT	1 mT	5 mT	10 mT
Progressive motility*	61.07 ± 8.87 62 (45–77)	15.22 ± 4.71^{a} 14 (10–25)	17.75 ± 6.36^{a} 16 (11–30)	18.57 ± 4.23^{a} 19 (10–23)	20 ± 14.23^{a} 20 (2–48)
Total motility*	87.92 ± 7.64 89 (72–97)	45.66 ± 9.11^{a} 45 (29–58)	47.7 ± 10.63^{a} 45 (31–70)	57.5 ± 10.94^{a} 58 (42–74)	63.44 ± 9.15 65 (45–77)
Viability*	89.57 ± 9.78 94 (71–99)	49.4 ± 14.15^{ba} 48 (32–68)	50.54 ± 6.57^{ba} 50 (42–64)	61.75 ± 7.44^{a} 64 (48–69)	69.36 ± 6.96 70 (58–83)
Normal morphology	15.34 ± 2.35	6.34 ± 1.25^{a}	7.26 ± 2.11^{a}	7.34 ± 2.65^{a}	8.14 ± 1.35^{a}
Intact acrosome	72.27 ± 12	56.91 ± 13.06^{a}	50.1 ± 9.19^{a}	46.66 ± 7.21^{a}	44.75 ± 11.12^{a}

*The data are presented as mean ± SD; Analysis was performed by One-way ANOVA with Tukey. *: The data are presented as mean ± SD, median (min–max); Analysis was performed by Kruskal-Wallis with Dunnett. ^{a}: significant compared to before freezing, ^{b}: significant compared to 10 mT.*

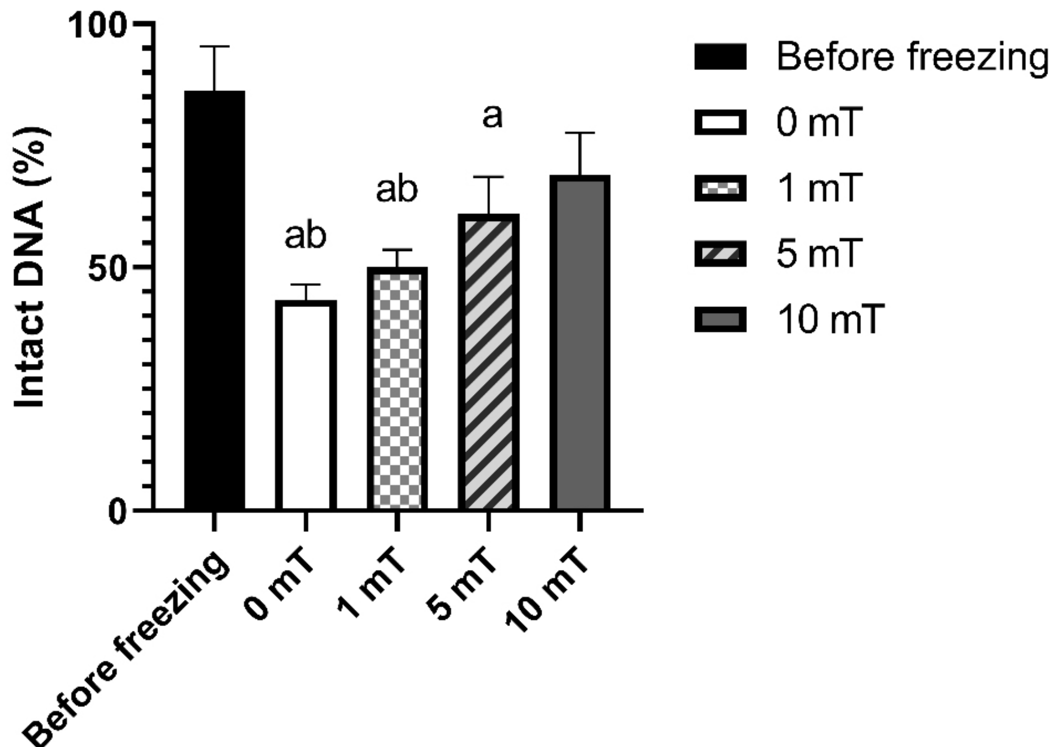

FIGURE 1. Comparison of sperm DNA fragmentation between different groups. a: significant compared to before freezing, b: significant compared to 10 mT. Analysis was performed by One-way ANOVA with Tukey.

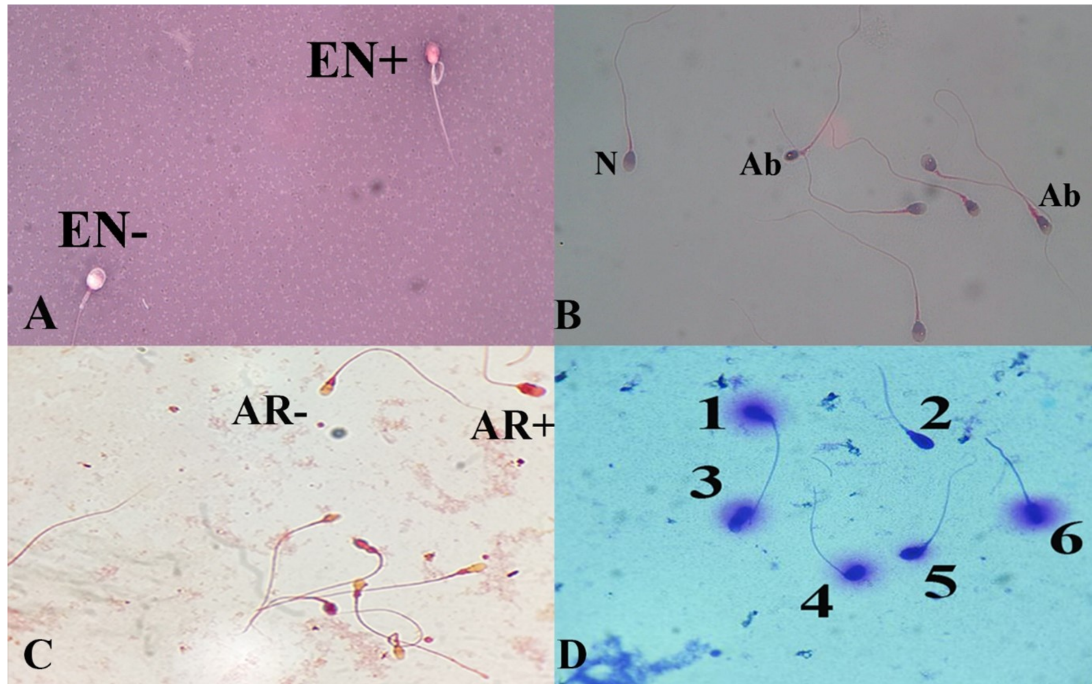

FIGURE 2. Evaluation of sperm viability, morphology, acrosome reaction and DNA fragmentation. (A) Sperm viability using eosin-nigrosin staining method, live (unstained) cell (EN−), dead (red) cell (EN+); (B) Sperm morphology using Papanicolaou staining, N: Normal, Ab: Abnormal; (C) Double staining test for evaluation of acrosome reaction, Cells with acrosome reaction (AR+), cell with intact acrosome (AR−); (D) Sperm chromatin dispersion test to evaluate sperm DNA fragmentation, 1: Normal cell with large halo, 2: Abnormal cell without halo, 3,4: Normal cell with medium halo, 5: Abnormal cell with small halo, 6: Normal cell with large halo (1000×).

investigated this effect on mouse oocyte freezing and showed that the destructive effects of freezing were reduced with the application of a SMF [22]. Magnetic fields can have biophysical effects on cells. In general, the interaction between the magnetic field and the biological system considers various parameters, such as the cell sample and the strength of the magnetic field. Magnetic fields can easily enter biological systems and cells and directly affect mobile charges, such as ions and proteins [23]. In general, magnetic fields are divided into four categories: weak (<1 mT), medium (between 1 mT and 1 T), strong (between 1 T and 5 T) and very strong (T >5) [24]. It is known that moderate-intensity magnetic fields are effective on biological systems. Although the magnetic position of the water molecule in cells can be oriented in the presence of a magnetic field, this possibility does not occur in cases such as our study, where low intensities of the magnetic field were used. The magnetic field required to induce magnetic moments and their rotation must be strong (>10 T). Therefore, weak or moderate magnetic fields have little effect on the magnetic dipolarization of water [25]. However, the application of a moderate SMF increases the surface tension and viscosity of the water. As a result of the increase in viscosity of water, the possibility of the formation of ice crystals is infinitesimally small, which leads to a reduction in cold damage [26].

Based on a study conducted by Formicki *et al.* [14] on the effect of a SMF (1–5–10 mT) for 24 hours on sperm movement parameters of Hochen Danube fish, they concluded that short-term storage of semen with a magnetic field has an effect on sperm movement parameters and increases the speed of sperm. As a result, it increases the probability of conception and fertility [14]. According to another study conducted by the same group, the magnetic field increases the speed of sperm, the chance of conception and fertility in salmon [13]. In a study where Ghafelebashi *et al.* [26] used moderate intensities of SMF and evaluated human spermatozoa for up to 5 hours, no adverse effects on the motility and viability of spermatozoa were observed. Therefore, it does not seem like the magnetic field has negative effects on sperm parameters in the short period that the sperm samples were exposed to the field in our study. The biological effects of magnetic fields are reversible [27]. However, it seems that the effects of SMF on cells may be reversible, depending on the intensity, duration, frequency and cell type.

So far, various mechanisms have been known to describe the biological effects of moderate SMFs. Cell membrane phospholipids act as a major factor in the interaction between the magnetic field and the quality of cells after freezing. Several studies have shown that the bilayer orientation of phospholipids can be changed following a SMF [28]. Due to their non-spherical shape, phospholipids behave as highly diamagnetic anisotropic molecules that can be oriented by the magnetic moment. Therefore, the diamagnetic anisotropy of the cell membrane increases. This modification is sufficient to cause a slow reorientation of the phospholipids. Membrane stiffness is accordingly increased while membrane fluidity and permeability are significantly reduced [29].

The mechanisms by which the magnetic field affects the freezing process are not fully understood. One hypothesis pre-

viously proposed by Rosen is that the application of SMF may cause magnetic reorientation of membrane phospholipids, particularly the acyl chains of the molecules, through diamagnetic anisotropy effects, which can affect membrane permeability. It has also been reported that magnetic fields can accelerate the release of water from cells and tissues, thereby preventing the toxic effects of cryoprotectants [28]. One reason why exposure to a SMF can effectively change sperm motility is because it alters the permeability of the sperm cell membrane. A chain of single-domain magnetic particles can detect the direction and intensity of magnetic fields. These particles are connected by microtubule-like strings with many ion channels in the cell membrane. Movement of the position of magnetic particles can cause opening and closing of the ion channels through the microtubule-like strings. This results in a change in the cell membrane potential and its permeability [29].

Blesbois *et al.* [30] conducted an experiment to freeze bird sperm and found that sperm with low membrane fluidity had a better survival rate after freezing. This study suggests that spermatozoa with less fluid membranes show more resistance to the stresses caused by freezing [30]. Lin *et al.* [10] also showed that red blood cells exposed to a SMF displayed greater membrane stability and resistance to a very low water environment in the dehydration stability test. Therefore, they concluded that exposure to a SMF reduced the fluidity of the red blood cell membrane, leading to two possible mechanisms beneficial for red blood cell coagulation. First, the decrease in membrane fluidity leads to a decrease in the base membrane's permeability. Second, increasing membrane hardness has a positive effect on membrane resistance against stresses caused by excessive dehydration. The positive results of using a magnetic field have led to the suggestion that the concentration of anti-freezing materials can be reduced [31]. Baniasadi *et al.* [22] showed that the membrane is the destination of the magnetic field. They used scanning electron microscopy to find that the membrane in the frozen group exposed to SMF suffered less damage than the control group. They also demonstrated that the application of a medium-intensity magnetic field increases the viscosity of water. Based on this, it can be concluded that the number of oocyte vacuoles was less in the SMF group [22]. The intensity of the field is one of the most important factors influencing the response.

A study conducted by Tablado *et al.* [11] examined the effects of a SMF with an intensity of 0.7 Tesla on the morphology and morphometry of epididymal sperm in mice. The study found that the motility of the epididymal sperm was not affected by the magnetic field, but an abnormality in the sperm head was observed, as the hook was absent [11]. The use of higher intensities can change the morphology, which is reversible [10], however, in low intensities, such as those used in our study, the SMF did not affect the morphology of the sperm during the freezing-thawing process. Ghafelebashi *et al.* [26] also did not observe any effect on the morphology of the sperm even when using low intensities of the magnetic field for 5 hours in *ex vivo* conditions. However, no effect on cell morphology was found when Khodarahmi *et al.* [32] applied 1/2 T to rat astrocytes for 72 hours, particularly, no membrane protrusions or changes in the surfaces. Therefore, it seems that even higher magnetic field intensities and longer times cannot affect cell morphology.

Recent studies have shown that magnetic fields increase cell membrane permeability, thereby increasing intracellular calcium concentration. This leads to increased cell viability and decreased cell apoptosis [33]. The extent of this effect depends on various factors such as cell type, intensity and duration of field radiation, permeability of tissues and other test conditions [24].

The results of this study indicate that, contrary to expectations, the protective effect of a SMF on sperm acrosome reaction was not observed as the intensity of the field increased. Instead, it was observed that with an increase in field intensity, the acrosome reaction increased. One of the phenomena caused by phospholipid reorientation is the change in membrane ion flux. The SMF mildly affects ion channels that arise from multiple protein subunits. Therefore, a moderate intensity field may stimulate the activation or inhibition mechanism of calcium channels. This hypothesis has been supported by the measurement of calcium flux during exposure to a SMF, which showed that SMF with an intensity of 6 mT in human lymphocytes causes an anti-apoptotic effect, an increase in the concentration of intracellular calcium, and diversity in the expression and distribution of anti-apoptotic and pro-apoptotic genes [34]. The SMF is also expected to change intracellular calcium concentration through endoplasmic reticulum stimulation [22]. Considering that increased intracellular calcium is associated with acrosome reaction, this increase in calcium may be a factor that increases the acrosome reaction with an increase in field intensity.

The high amount of unsaturated fatty acids in the sperm plasma membrane and the loss of a large volume of cytoplasm during maturation lead to the presence of a weak antioxidant system in the sperm. During the freezing-thawing process, the amount of reactive oxygen species (ROS) increases in the sperm cell. Previous studies are contradictory about the effect of the SMF on ROS. Long times of exposure of the cell with the SMF lead to an increase in ROS, while short times do not cause a change or lead to a decrease in ROS [19]. The exposure time to SMF was not high enough to alter the ROS levels. However, in the current study, the level of ROS was not investigated, and this is one of the limitations of this study.

The results of the present study showed that SMF with an intensity of 10 mT was effective in reducing the amount of DNA fragmentation. Previous studies have shown that exposure to a SMF (1, 5 and 10 mT) in the short term does not cause DNA fragmentation in salmon sperm heads [13]. Additionally, exposure to a SMF can control DNA damage and/or damage repair through mitochondrial mechanisms [35]. In fact, mitochondria play a vital role in sperm function and motility. Previous studies have also shown that exposure to low SMF intensities cannot damage sperm DNA [36].

Regarding the clinical use of this method, it can be mentioned that based on the data from this research, it is still not possible to recommend the use of this method in the clinic. However, using other intensities in the range of average intensity of the SMF may be a solution in this field; for example, an intensity between 5 and 10 mT can be measured. Our study evaluated only normozoospermic samples. Abnormal sperm samples may react different when are exposed to SMF which

could be matter of ongoing researches. The possibility of using the field in all stages of the freeze-thaw process and checking the amount of ROS following the use of the field are among the factors that should be evaluated in further researches. More studies should be conducted so that this method can eventually become a common and widely used technique in infertility treatment centers to preserve all sperm parameters during the thaw-freezing process.

5. Conclusions

In summary, it can be concluded that using a SMF with a dose of 10 mT can have a protective effect on the parameters of motility, viability and DNA fragmentation during the freezing-thawing process.

AVAILABILITY OF DATA AND MATERIALS

The data presented in this study are available on reasonable request from the corresponding author.

AUTHOR CONTRIBUTIONS

IH, PA—designed the research study. NKD—performed the research; wrote the manuscript. IH—analyzed the data. All authors read and approved the final manuscript.

ETHICS APPROVAL AND CONSENT TO PARTICIPATE

Prior informed consent was obtained from all men and the ethical committee of the authors' institution approved this study (IR.MODARES.REC.1399.118).

ACKNOWLEDGMENT

This paper was extracted from MSc thesis of NKD. The authors would like to thank Behnam Hajipour for his great help during this study.

FUNDING

The authors thank Tarbiat Modares University for financial support (grant number: MED84185).

CONFLICT OF INTEREST

The authors declare no conflict of interest.

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How to cite this article: Negin Kargar Dahr, Parviz Abdolmaleki, Iman Halvaei. Static magnetic field can ameliorate detrimental effects of cryopreservation on human spermatozoa. Revista Internacional de Andrología. 2024; 22(2): 27-34. doi: 10.22514/j.androl.2024.012.