Original Article

Comparative Study of the Effect of Sperm-Cryoprotectant Media on Post-Freezing Sperm Quality in Oligoasthenoteratozoospermia (OAT) Males

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Background: Sperm cryopreservation is one of the important processes of reproductive technology. Because of the nature of the frozen-thawed process, the sperm are subject to being damaged in its structure and function after the thaw, especially in men who have shown an abnormal sperm-analysis result. They are more likely to have damaged sperm DNA as compared to normozoospermia men.

Objective: The present study aims to compare the efficiency of the sperm cryoprotectant media with four types of complex cryoprotectant agents (or CPA, namely GEYC, SF, TYB, and HSPM) to prevent sperm DNA damage in men who have oligoasthenoteratozoospermia (OAT).

Materials and Methods: Semen samples of the 50 OAT men were divided into 5 groups, namely group 1: prior-freezing sperm (Control), group 2: frozen sperm by glycerol egg yolk citrate (GEYC), group 3: frozen sperm by Sperm freeze (SF), group 4: frozen sperm by TEST-yolk-buffer (TYB) and group 5: frozen sperm by human sperm preservation medium (HSPM). The dissolved sperm was evaluated by sperm quality, as measured in the percentage of sperm motility, normal sperm morphology, vitality, and sperm DNA damage. The data consisted of a comparison between sperm before freezing and sperm in each of the frozen-thawed groups.

Results: The comparison of the frozen sperm (in groups 2, 3, 4 and 5) with the control group showed that the percentage of sperm motility, normal sperm morphology, and sperm vitality had significantly decreased. Furthermore, the rate of sperm DNA damage had significantly increased in the frozen sperm. When comparing the dissolved sperm properties between the four groups of CPAs, there was no difference in sperm vitality, motility, and normal sperm morphology. In terms of sperm DNA damage, the sperm in the GEYC and SF groups had less DNA damage than in the HSPM and TYB groups. However, there was no statistically significant difference between the GEYC and SF groups regarding DNA damage.

Conclusion: In the infertile men who have OAT, freezing sperm with GEYC or SF helps to reduce sperm DNA damage, as compared with sperm that has been frozen by HSPM and TYB. The research data suggest that sperm of infertile men who have OAT should be frozen using the cryoprotectant composed of egg yolk because of its ability to reduce sperm DNA damage when taken from the freezing procedure.

Keywords: Oligoasthenoteratozoospermia, Sperm cryopreservation, Sperm DNA damage

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Male infertility is a common problem and an important part of infertility therapy. Any abnormalities in human spermatozoa production and function in the presence of other factors account for 35 to 50% of all infertility cases[1]. One instance of male infertility presentation consists of oligoasthenoteratozoospermia (OAT), which is defined as being below a lower reference limit of the total number of or concentration of spermatozoa (<15x10^6/ml), motility <40%, percentage of progressively motile (PR) (<32%) and morphologically normal spermatozoa (<4%)[2].

Sperm cryopreservation is one of the essential parts of reproductive treatment essential to maintaining the quality of frozen sperm. According to the freeze and thawing process, the sperm are at risk of injury to its structure and function after the thawing. In the early stages of development, this technology affects the damage of sperm motility and viability with considerable variation between the ejaculates of different individuals[3-5]. There is some reported lower conception rates in the artificial insemination of frozen-thawed donor semen[4]. However, men with infertility have a greater tendency to decline in their post-thaw sperm quality than do

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normozoospermia men, especially in sperm DNA integrity[6].

There are currently three methods of cryopreserved sperm: slow, rapid, and programmable freezing; liquid-nitrogen vapor freezing; and vitrification. Regardless of the sperm-freezing technique being implemented, it is necessary to add the cryoprotectant in the processed semen sample. A cryoprotectant has a low molecular-weight chemical composition that helps to prevent ice formation in sperm cells, optimize osmotic pressure and pH, and provide extracellular energy to sperm[6-8].

Cryoprotectant agents (CPA) can be divided into two classes: 1) permeating CPA: these substances can cross through the cell membrane and maintain intracellular and extracellular solute concentrations at levels that the cell can tolerate and include glycerol, dimethyl sulfoxide (DMSO) and ethylene glycol; and 2) nonpermeating CPA: the active mechanism of these CPAs is partial cell dehydration and stabilization of the cell membrane; included among these CPAs are sucrose and glycine[3,4]. The commonly used CPAs are ethylene glycol and glycerol; but, in the use of glycerol, caution must be exercised because of its toxic effect[3,6]. Using an excessive glycerol concentration will cause the sperm cells to become damaged by osmotic injury while dissolving[6]. To improve the survival rate, more complex diluents containing other mainly nonpermeable cryoprotectant agents, such as glycine, zwitterions and egg yolk, were developed[3]. There are two groups of complex cryoprotectant agents: 1) egg yolk solution: glycerol egg yolk citrate (GEYC) and test-yolk-buffer (TYB) are a mixture of many CPAs. GEYC contains glycerol and egg yolk to protect the sperm cell membrane and prevent oxidative stress within cells[3,7]. The TYB solution was developed from the TESTY solution since it contains two of the Zwitterionic buffers, TES [N-tris (hydroxymethyl), methyl-2-aminomethane sulphonic acid] and TRIS [tris (hydroxymethyl) aminomethane] (which is popularly called "TEST" mixed with egg yolk and sodium citrate without glycerol). However, it is currently produced in a form that contains glycerol. 2) Non-egg yolk solutions: this human sperm-preserving medium (HSPM) is a sperm-freezing solution that has been developed from the Tyrodes medium consisting of many CPAs, such as glycerol, sucrose, glucose, glycine and human serum albumin (HSA) and HEPES (N-(2-hydroxyethyl) piperazine-N '(2-ethane) sulphonic acid), but without egg yolk[3,7].

The purpose of this study was to compare the efficiency of the sperm cryoprotectant media with four types of complex cryoprotectant agents (GEYC, SF, TYB, and HSPM) with the goal of preventing sperm DNA damage in men who have the OAT condition.

Materials and Methods

The experimental study was conducted at the Thammasat Fertility Center, Thammasat University Hospital. Approval from the Ethics Committee of the Faculty of Medicine, Thammasat University, was obtained prior to the study (MTU-EC-OB-1-200/60). Informed consent for participation was obtained. Subjects were recruited from the male partners of the infertility couples attending the Thammasat Fertility Center during February 2018 to March 2019. The participants were men who had a semen-analysis result compatible with the OAT condition, which is defined as a concentration of spermatozoa <15x10^6/ml, motility <40%, progressively motile <32%, and morphologically normal spermatozoa <4%[2].

The exclusion criteria were taken from participants who had reproductive-system infections, such as the human immunodeficiency virus (HIV), syphilis, gonorrhea or a hepatitis B infection. Participants who drank and smoked, including those who had prolonged contact with medications and were regularly taking herbal medicines and participants who did not abstain from semen ejaculation for a specified period of 2 to 7 days were both excluded.

Prior-Freezing Semen analysis

Participants collected a semen sample in a sterile plastic container using a masturbation method. A sperm-quality analysis was then conducted. After seminal liquefaction, a routine semen analysis was performed to determine sperm normal morphology and motility according to World Health Organization specifications[2].

Division of Semen samples

The semen sample of each participant was separated into units of 250 µl per group as follows.

1) Control group or group with prior-freezing semen (control).

2) Frozen-sperm group with GEYC solution (freezing medium consisting of 10% concentration of GEYC produced by the Reproductive Medicine Unit, Department of Obstetrics and Gynecology, Faculty of Medicine, Chulalongkorn University).

3) Frozen-sperm group with Sperm Freeze (SF) solution (freezing medium, Kitazato Biopharma, Shizuoka, Japan).

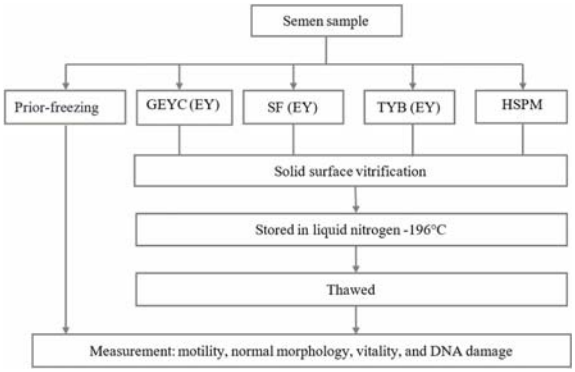
4) Frozen sperm with a TYB solution (freezing medium, Irvine Scientific, Irvine, CA).

5) Freezing sperm with HSPM (freezing medium, FertiPro N.V., Beernem, Belgium).

Samples from all of the experimental groups underwent the frozen-thawed process. The sperm were then examined, first by a semen analysis (SA) to detect motility and normal morphology, then by the hypo-osmotic swelling test (HOS) to measure live sperm rate (vitality) and, finally, by the comet assay to measure DNA damage (same method as in the pre-freezing semen) (Figure 1).

Sperm Frozen-Thawed process

The freezing of the sperm took place by the solid-surface vitrification technique. The process was started by putting liquid nitrogen into a foam box, filling about half of the box. Aluminum-foil paper was then folded into a circular cup shape with a diameter equal to the size of the foam box. Its depth was about 1 cm. The aluminum foil paper was inserted into the foam box. The foam container was closed to



EY = Egg yolk containing cryoprotectant agent

Figure 1. Diagram demonstrating the experimental procedure.

allow the temperature of the aluminum paper to approach the liquid-nitrogen temperature (-196°C). The semen was then mixed with the cryoprotectant media at a ratio of 1: 1 (V/V) and an autopipette was used to draw the 20 µl mixture quickly into the foil until the mixture is exhausted. All components of the frozen sperm were round, hard and white. The frozen sperm were stored in a cryovial (Nunc, Thermofisher Scientific, MA). The frozen sperm was then soaked in a liquid-nitrogen tank at -196°C for about 7 days.

Thawing of the sperm was performed by taking the freezing cryovial from the nitrogen tank to dissolve it. Sperm tablets were poured onto a plastic plate to culture the embryo. The frozen sperm granules were separated apart. They were then left to dissolve for about 4 minutes. The autopipette was then used to absorb the dissolved sperm in the 5 ml round-bottom tube. The sperm was then taken to be examined for sperm motility, normal morphology, HOS and Comet assay[9].

Sperm motility

The sperm were examined for motility by dropping the semen sample onto the Makler counting chamber and then counting it with the machine Computer-Aided Sperm Analysis (CASA).

Sperm morphology

About 20 µl of semen was dripped to form thin smears on a clean slide glass and was then left to dry on a warm platform for about 30 seconds. These steps then followed: The slide was dipped in absolute methyl alcohol (methanol) for 20 seconds and then dipped in eosin for 20 seconds. It was washed in distilled water for 10 seconds and the slide was then dipped in methylene blue for 20 seconds and washed with distilled water for 10 seconds.

The dyed slides were examined under the microscope, first with low magnification, and then with a higher magnification. Viewing requires a 100x magnification of the objective lens. An oil-immersion drop is needed on the slide area for observation under the camera.

The sperm-morphology number was counted in 100 cells by use of the white-blood-cell counting machine. The percentage of the normal and irregular morphology of each type was determined by the use of the WHO 2010 specifications[2].

Sperm vitality

The hypo-osmotic swelling test (HOS test) is an alternative sperm vitality test used whenever the sperm staining test is avoided[2]. The test is used to determine the number of live sperm by use of the principle of osmotic pressure. The method involves putting the sperm into a mixture with a hypo-osmotic solution containing sodium citrate, fructose, and distilled water. A 0.5 ml of undiluted semen was mixed with 1 ml of the hypo-osmotic solution and incubated at 37°C for 30 minutes. Drops of the solution were then laid onto the slide and covered with a coverslip. The hypotonic solution is capable of penetrating into the cell membrane of the live sperm, causing the tip of the tail to expand, swell and curl. A total of 100 spermatozoa are then counted with three repetitions under a phase-contrast microscope to calculate the sperm-vitality rate[10].

Sperm DNA damage

Single-cell gel electrophoresis of sperm DNA (Comet assay) was performed as previously described[11]. Preparation took place at the normal melting point on an agarose slide and sperm were then thawed at room temperature. The dissolved sperm was diluted in PBS to $5x10^6$/ml and an aliquot of 10 µl was then mixed with 90 µl of low melting-point agarose and placed on the normal melting-point agarose slide. The slides were transferred to a 4°C medium to promote solidification. The top layer was performed by dropping the normal melting-point agarose on top of the previous two layers of the sample slide, resulting in three layers of gel on the slide with a sample of spermatozoa in the middle layer. The sample slides were submerged in the lysis solution (Tris 10 mmol/l, 0.5 mmol/l EDTA and 2.5 mol/l NaCl, pH 10) contained 1% Triton X-100) and was contained with Dithiothreitol (DDT) for 2 hours. The electrophoresis was performed at 24V, 300 mA for 20 minutes at 4 degrees Celsius and then washed by the neutralized buffer for 5 minutes. An ethidium bromide solution was dropped onto the sample slide and covered by the coverslip. Examining the sample slide under the fluorescence microscope involved at least 100 spermatozoa for each sample, while the tail length was analyzed by the meta-system software, comet imager version 2.2. The results are shown in Figure 2.

For the purpose of data and statistical analysis, the baseline characteristics of sperm before and after the frozen-thawed process were compared. Sperm motility, normal morphology, vitality and DNA damage were reported as percentages. The unpaired T-test was used to compare the mean values, with a standard deviation of percentages among groups.

Results

A total of 50 OAT males were enrolled during the period under study, providing complete data for analysis. The mean age of the participants was 36.67 years. The means and standard deviations of sperm characteristics, including semen volume, pH and sperm concentration, were 2.96±1.36 ml, 8.02±0.1, and 5.155±2.94 10^6/ml, respectively. The mean percentages of sperm motility, normal morphology and vitality rate of the pre-freezing semen were 21.73±11.06, 1.78±1.09 and 43.88±15.85, respectively.

Prior-freezing semen was compared with the post-frozen-thawed semen in four types of cryoprotectant agents (CPA), as shown in Table 1. The mean percentage of sperm motility had significantly decreased in all CPA media, as compared with the prior-freezing semen (p<0.05). Sperm motility in GEYC media had the lowest percentage (7.36±5.72), while the sperm motility in HSPM media had the highest of mean percentages (8.77±4.86) among all CPA media.

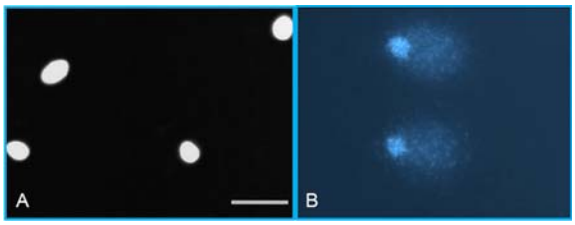
In the comparing of normal sperm morphology, the mean percentage had a significant decrease in all four CPA groups compared with the pre-freezing sample (p<0.05). While there was no significant difference among the four media, the normal morphology in GEYC media was found to have the highest mean percentage (1.52±1.30).

In comparing sperm vitality, the mean percentage had a significant decrease in all four CPA groups as compared with the pre-freezing sample (p<0.05). However, there were no significant differences between the four media. The sperm vitality rate in HSPM media had the highest mean percentages (21.02±9.56).

In terms of sperm DNA damage, the post-frozen-thawed sperm from all four types of CPA had significantly enhanced DNA damage (p<0.05), as compared with the pre-freezing sample. However, sperm DNA damage in TYB and HSPM media had significantly higher values than in the GEYC and SF media (50.68±12.93, 50.70±11.26 vs. 47.10±12.99, 46.63±13.72, respectively with p<0.05), as shown in Figure 3.

Discussion

Accordingly, the frozen-thawed process causes



Source: The Fertility Center, Thammasat University Hospital.

Figure 2. Images demonstrating results of the Comet assay. (A) is the sperm with no DNA damage, and (B) is the sperm with DNA fragmentation.

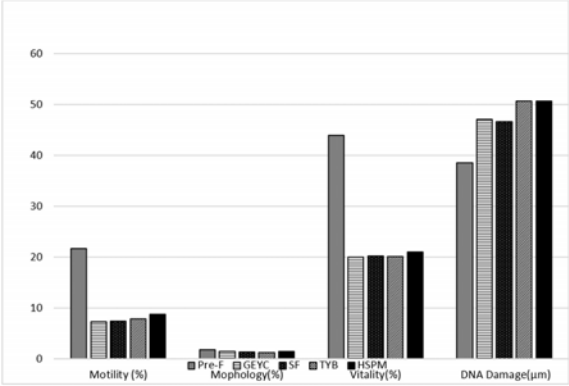


Figure 3. The comparison of the quality of sperm before and after the frozen-thawed process in four different Media.

Table 1. Comparison of the quality of sperm characteristics before and after the frozen-thawed process in 4 types of CPA

Group	Sperm characteristics			
	Motility (%) (mean ± SD)	Normal morphology (%) (mean ± SD)	Sperm vitality (%) (mean ± SD)	DNA damage (%) (mean ± SD)
Prior-freezing semen	21.73±11.06	1.78±1.09	43.88±15.85	38.51±10.07
Frozen-Thawed				
GEYC	7.36±5.72[a]	1.52±1.30[a]	20.06±5.65[a]	47.10±12.99[a]
SF	7.40±5.12[a]	1.40±0.99[a]	20.22±10.21[a]	46.63±13.72[a]
TYB	7.84±5.75[a]	1.30±1.22[a]	20.18±10.40[a]	50.68±12.93[a,b,c]
HSPM	8.77±4.86[a,b]	1.46±1.28[a]	21.02±9.56[a]	50.70±11.26[a,b,c]

[a] = Compared with the prior-freezing semen, with significance at p<0.05
[b] = Compared with GEYC group, with significance at p<0.05
[c] = Compared with SF group, with significance at p<0.05

injury to sperm structure and function, and affects the sperm both physically and biochemically, whether in the form of a decrease in movement, morphology or vitality, including changes in the plasma membrane, acrosome, and sperm DNA integrity[5,6,10,11]. These detriments are all related to the decrease in fertilization rate and pregnancy rate[12,13]. In the previous studies, it was found that the sperm of infertile men had post-thawed properties lower than normozoospermia because those sperm are more susceptible to destruction[5,10,11,14]. The fertilization rate of sperm has been frozen in relation to the initial quality of sperm selection, the CPA and the technique of freezing and thawing, as well as the percentage of sperm DNA damage[3,4,6].

Vitrification is a technique developed to minimize the risk of the destruction of cells by the freezing process. It avoids the occurrence of dangerous chilling injuries caused by freezing cells by the rapid reduction of temperature in seconds and avoids the occurrence of ice crystals within the cell by changing the solution inside the cell into a glassy solid[7,8]. The present study used solid-surface vitrification (SSV), which is a quick-frozen technique that uses a sample of cells exposed to low temperatures near the temperature of liquid nitrogen at a low point. The sample cells will contact the metal or cold-surface material at a temperature of -196°C. The material must have enough space for the tissue or cell to undergo a rapid temperature reduction rate and avoid nitrogen bubbles and evaporation. By not reducing the cooling rate, this method has the advantage of saving time, cost and can freeze sperm in small volumes[7,9].

During the cryopreserve process, about 25% to 75% of the sperm will be destroyed, and the most common cause of this destruction is the occurrence of oxidative stress (OS) and the formation of intracellular ice crystals[5,6]. Cryoinjury is usually affected by sperm vitality and movement[5]. Hammadeh et al found that the sperm cryopreservation causes significant damage to the sperm normal morphology and membrane integrity, especially to the sperm head and the tail, resulting in significantly decreased sperm motility in both fertile men and infertile men[10]. Consistent with the present study was the discovery that, in all cryopreserved groups, the post-thawed sperm properties, including motility, normal morphology and vitality, decreased significantly as compared with the pre-freezing sperm. In terms of sperm motility, the present study found that the mean percentage had the highest after-freeze-thaw with HSPM, but at levels that were not statistically significant. This outcome corresponds to the study of Centola et al, who found that the HSPM had significantly higher motility in the normozoospermia men than either the TYB or glycerol[4]. However, the present study had the opposite outcome in Hammadeh's study, which found that the TYB had a higher mean percentage of post-thawed sperm motility in the infertile men than did the HSPM[15]. The present study found that the mean percentage of sperm morphology, which had the lowest after freeze-thaw with TYB, conflicted with the outcome in Hammadeh's study, which found that TYB had a higher mean percentage of post-thawed normal sperm morphology, as compared with HSPM[15].

Sperm chromatin condensation of the infertile men had more deterioration, which reflects the fact that the infertile men had less ability to withstand the cryoinjury than normozoospermia men[6,9,10,15]. Shamsi et al assessed the sperm DNA damage after cryopreservation by the comet assay technique in five categories of infertile men, including oligozoospermia (O), asthenozoospermia (A), teratozoospermia (T), OAT, and infertile men with a normal semen analysis. The result found that the OAT group had the highest rate of post-thawed sperm DNA damage[16]. The cold shock and the atmospheric oxygen undergo a change in the cryopreservation process, leading to the rapid production of reactive oxygen species (ROS)[6]. Kalthur et al found that abnormal morphological sperm may be a source of free radicals. The amount of ROS increases during the freeze-thawed process of abnormal sperm and may be higher than normal sperm, leading to a level of DNA damage that increases in an abnormal sperm[17].

Although the sperm-DNA damage in the present study significantly increased in all the cryopreserved groups, the sperm which froze by GEYC and SF were significantly less in their mean percentage of post-thawed DNA damage than the two other CPAs. However, the mean percentage of DNA damage in the TYB group was less than in the HSPM group event if it was not statistically significant. Nonetheless, it was consistent with Hammadeh et al whose findings supported the fact that TYB preserved chromatin and morphology significantly better than did HSPM[15]. According to GEYC and SF, the solutions involved consist of CPA egg yolk-containing compounds that contain cholesterol, phospholipid, and antioxidants. This CPA may help protect sperm DNA damage from occurrences of osmotic shock[3,6].

Conclusion

Infertility men who have OAT tend to have more sperm DNA damage, especially when sperm cryopreservation is indicated. Therefore, sperm cryopreservation of infertile males with OAT should be implemented using CPA with an egg yolk component that will help reduce sperm genetic material deterioration and increase the success rate of infertility treatment outcomes. This research was limited in the number of abnormal semen samples collected and may not have been enough to make a statistically significant difference at any point.

What is already known on this topic?

Previous research has involved studies aimed at improving the sperm-freezing technique. Past studies have included pre-frozen sperm preparation, post-thawing processes and the choice of CPAs, as well as the other conditions of the freezing and thawing processes. By studying both normal sperm and abnormal sperm samples, it was found that sperm freezing in males with infertility caused by abnormal sperm is more likely to cause damage to genetic material (DNA) and the changes in the structure of chromatin during the freezing and thawing process than in

normozoospermia men.

What this study adds?

The present study found that the use of CPAs which contain glycerol and egg yolk in the main components can help to prevent the occurrence of sperm genetic damage. Therefore, these CPAs are suitable for use in the process of infertility treatment in couples of whom the male partners have abnormal sperm quality.

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Potential conflicts of interests

The authors declare no conflicts of interest.

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การศึกษาเปรียบเทียบผลของน้ำยาแช่แข็งอสุจิต่อคุณภาพอสุจิหลังการละลายในผู้ชายที่มีอสุจิผิดปกติ Oligoasthenoteratozoospermia (OAT)

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ภูมิหลัง: การแช่แข็งอสุจิมีความสำคัญทางการแพทย์และเทคโนโลยีช่วยการเจริญพันธุ์ จึงมีความพยายามพัฒนาปรับปรุงเทคนิคและวิธีการแช่แข็งเพื่อรักษาคุณสมบัติของอสุจิหลังการแช่แข็งให้ได้ผลดีที่สุด เนื่องจากกระบวนการแช่แข็งมีโอกาสเกิดอันตรายต่อโครงสร้างและการทำงานของอสุจิ โดยเฉพาะอสุจิของผู้ชายที่มีภาวะมีบุตรยากมีแนวโน้มเกิดความเสียหายของสารพันธุกรรม (ดีเอ็นเอ) มากกว่าของผู้ชายที่มีคุณภาพของอสุจิปกติ

วัตถุประสงค์: เพื่อเปรียบเทียบประสิทธิภาพของน้ำยาแช่แข็งอสุจิที่มีส่วนประกอบของ cryoprotectant media 4 ชนิดในการป้องกันความเสียหายดีเอ็นเอของอสุจิที่มีความผิดปกติแบบ oligoasthenoteratozoospermia (OAT)

วัสดุและวิธีการ: กลุ่มตัวอย่างอสุจิที่มีความผิดปกติแบบ OAT จากอาสาสมัคร 50 คน นำอสุจิของแต่ละคน แบ่งออกเป็น 5 กลุ่ม คือ กลุ่มที่ 1: อสุจิก่อนการแช่แข็ง กลุ่มที่ 2: อสุจิที่แช่แข็งด้วยน้ำยา glycerol egg yolk citrate (GEYC) กลุ่มที่ 3: กลุ่มอสุจิที่แช่แข็งด้วยน้ำยา Sperm Freeze (SF) กลุ่มที่ 4: อสุจิที่แช่แข็งด้วยน้ำยา TEST-yolk-buffer (TYB) และกลุ่มที่ 5: อสุจิที่แช่แข็งด้วยน้ำยา human sperm preservation medium (HSPM) เมื่อแช่แข็งและละลายอสุจิแล้วตรวจประเมินร้อยละของการเคลื่อนไหว อสุจิที่มีรูปร่างปกติ อสุจิที่มีชีวิต และความเสียหายของดีเอ็นเอ นำผลมาวิเคราะห์เปรียบเทียบคุณสมบัติของอสุจิก่อนและหลังการแช่แข็ง และเปรียบเทียบผลของการแช่แข็งอสุจิในแต่ละกลุ่มน้ำยาแช่แข็ง

ผลการศึกษา: เมื่อเปรียบเทียบกลุ่มอสุจิที่แช่แข็งกับกลุ่มก่อนการแช่แข็ง พบว่าร้อยละของการเคลื่อนไหว อสุจิที่มีรูปร่างปกติและอสุจิที่มีชีวิต มีค่าลดลงอย่างมีนัยสำคัญทางสถิติ ความเสียหายของดีเอ็นเอเพิ่มขึ้นอย่างมีนัยสำคัญทางสถิติ เมื่อเปรียบเทียบระหว่างกลุ่มอสุจิที่แช่แข็งในน้ำยา 4 ชนิด ไม่พบความแตกต่างของร้อยละของอสุจิที่มีชีวิตและรูปร่างอสุจิที่ปกติ แต่พบว่ากลุ่มน้ำยา HSPM มีร้อยละการเคลื่อนไหวสูงกว่ากลุ่มน้ำยา GEYC อย่างมีนัยสำคัญทางสถิติ ในส่วนของการประเมินความเสียหายของดีเอ็นเอ ของอสุจิพบว่า กลุ่มน้ำยา GEYC และ SF มีความเสียหายของดีเอ็นเอน้อยกว่ากลุ่มน้ำยา HSPM และ TYB อย่างมีนัยสำคัญทางสถิติ แต่ไม่มีความแตกต่างกันทางสถิติระหว่างกลุ่มน้ำยา GEYC และ SF

สรุป: การแช่แข็งอสุจิของชายที่มีความผิดปกติของอสุจิแบบ OAT ด้วยน้ำยา GEYC และ SF ช่วยป้องกันความเสียหายของดีเอ็นเอได้ดีกว่าน้ำยา HSPM และน้ำยา TYB ดังนั้นการแช่แข็งอสุจิของผู้ชายที่มีภาวะมีบุตรยากควรใช้น้ำยาแช่แข็งที่มีส่วนประกอบของ Egg yolk เพราะจะช่วยป้องกันความเสียหายของดีเอ็นเอจากกระบวนการแช่แข็งได้ดี