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Study of qualitative and quantitative indicators of the spermatogenesis for determination of the effectiveness of cryopreservation

Abstract: Semen or spermatozoa cryopreservation (commonly called sperm banking) is a procedure to preserve sperm cells. Semen can be used successfully indefinitely after cryopreservation and might be stored successfully over 20 years. It can be used for sperm donation where the recipient wants the treatment in a different time or place, or as a means of preserving fertility for men undergoing vasectomy or treatments that may compromise their fertility, such as chemotherapy, radiation therapy or surgery. However, before the cryopreservation, it is necessary to check the parameters of the ejaculate and treat the sperm in a special container (a small in diameter plastic box with information about the patient) to increase the concentration of spermatozoa. Put the container for storage into Dewar tube. In some cases, cryopreservation might worsen the quality of the ejaculate. After freezing, the mobility and morphology of the sperm can deteriorate. Thus, it is desirable to carry out freezing in test conditions to determine the need for the frozen material. Especially when spouses plan to use additional fertilization programs, this approach should be taken into account, since cryopreservation might cause a spontaneous change in the program of intracytoplasmic vaccination of the spouse. One of the methodological problems of cryopreservation is poor quality of the ejaculate, i.e. when freezing spermatozoa, their activity and function deteriorate from the norm. This reduces the possibility of using them in the future for artificial insemination. Results of the experiment show that cryopreservation is possible only in specialized centers with the highest professional standards.
Key words: spermatogenesis, infertility, ejaculate, cryopreservation.

Introduction

Further conservation of spermatozoa by cryopreservation method is a complex process, requiring special responsibilities and putting potential staff liabilities. It is advisable to evaluate the impediments to the researcher's work. In most cases, it is necessary to clarify the data on spermatozoa cryopreservation by evaluating the patient's psycho-emotional state. In the first place, this information may have a negative impact on the patient's psychological state [1-3].

In 1776, Spallancani, the first to maintain their mobility after dissolving spermatozoa. In the mid-nineteenth century, the idea of freezing spermatozoids was used to breed cattle. Apparently, the possibility of freezing army's sperm to allow their wives to have children when they were killed in the war began to be considered [4]. Successful fertilization and pregnancy induction for the first time with cryoconcentrated spermatozoids began in the first half of the 20th century. However, the decrease in spermatozoa concentration is not the most important factor in predicting the success of artificial insemination [5].

Cryopreservation of spermatozoids before the treatment, leading to the disorders of patients with oncological diseases, is a chance to preserve their reproductive capacity.

Ejaculators can be stored according to the following indicators:

– Potentially sterile chemotherapy and radiation therapy for malignant neoplasm and non-tumor diseases;

– Before operations that can cause a sharp decrease in men's fertility;

– The quality of ejaculate, which can lead to the appearance of azoospermia, in men with a decline in quality [6];

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– In men with non-obstructive azoospermia, the probability of separating spermatozoa using spermatozoa techniques is about 60-70% [7-12];

– Cryopreservation can also be used to preserve spermatozoids in intracytoplasmic doses in an amount that would prevent additional pairing of the super ovulation of the sperm (female);

– For insemination, the procedure is for the administration of intracytoplasmic vaccine with her sperm in the absence of a new ejaculate [13-19].

Materials and methods

60 healthy men aged 25-40 have voluntarily agreed to undertake the study by filling the special consent form. The results were obtained on three main indicators, i.e morphology, mobility, and quantitative index of sperm.Quantitative and qualitative indicators of the results of the spermogram were evaluated according to the criteria of the World Health Organization (WHO). We compared and analyzed quantitative and qualitative indicators of 3 types of environment for freezing the sperm.

The initial macroscopic evaluation of the ejaculate was performed visually under a microscope and calculated by the Laboratory Cell Calculator (Macler Method) with observation carried out in a special chamber with depth of only 10 microns (1/10 of the depth of the cell for counting blood cells) between two flat panes. With the computer method for assessing the motility of spermatozoa, the complex has the following configuration: a microscope, a television camera, a block for entering a television signal into a computer, a computer, special software. Using a television camera, a video of the movement of spermatozoa is recorded in the computer in real time. Then the computer, based on the analysis of individual frames of the video, calculates the motility of the spermatozoa.

In order to protect the frozen spermatozoa antioxidants (vitamine E), calcium ions-related substances (ethylenediaminetetraacetic acid), as well as phosphatyltillicoline (platelet activating factor) or methylxanthin were used.

Results and discussion

Men aged from 31 to 35 years old and older than 40 years were detected in order to determine the number of sperm in the ejaculate. Men were divided into three groups. In the study, the results of the difference in the number of sperm and men in the 1 mL ejaculate were shown (Figure 1).

According to the findings, the maximum concentration of sperm (56.21 million) identified in the group of men aged 31-35 years. The average value of these indicators depends on the age range of 26-30 years. They were found in men group 36-39 years and over 40 years. The concentration of spermatozoa in men aged between 26 and 30 years in 1 mL ejaculate is 55.62 million, respectively. The concentration of sperm in 1 mL of ejaculate in men between 31-35 years is 53.62 million and the concentration of sperm in 1 mL of ejaculate in the male group of 40 years is 51.65 million tons. However, as a result of the expertise, the group with the most promising concentration of spermatozoids was men under the age of 25. The mean concentration of spermatozoids in this group was 39.68 million times in 1 mL ejaculate.

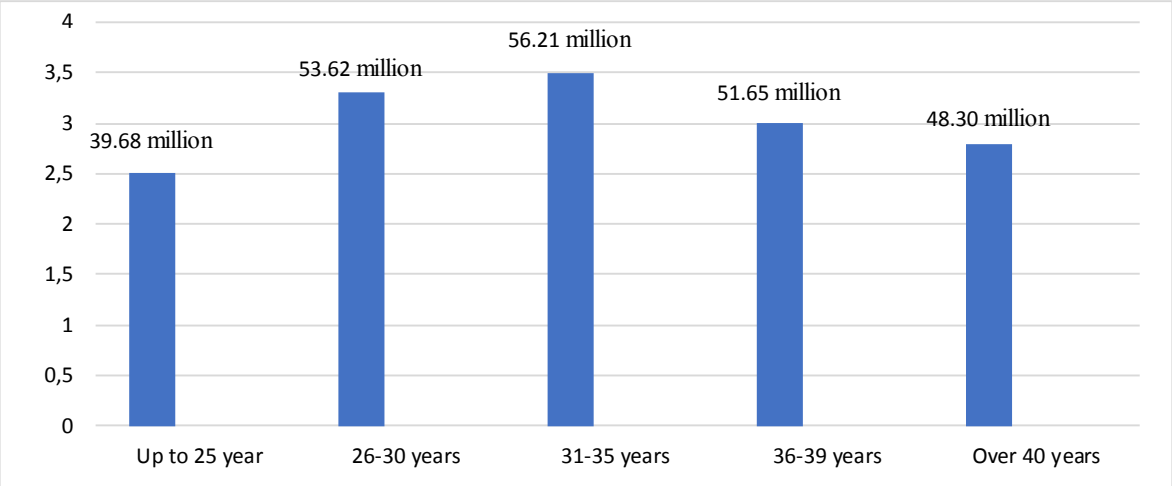

Figure 1 – The number of spermatozoids in 1 mL ejaculate of different ages of men

Similar results were obtained in the study of the share of active motion sperm in 1 mL ejaculation of men in different age groups. The best results were found in age groups between 36-39 and 31-35 (32.5% and 31.5% respectively), with a relatively small percentage of active motion spermatozoa fractions (18.4%) (Figure 2). The results of this series show that the lowest rates for sperm motions in active motion are typical for age groups up to 25 and 26-30 years (14.5% and 17.40%, respectively).

Morphologically normal spermatozoa were at lower WHO threshold levels in groups 31-35 and 36-39, respectively, and were 14.5% and 13.3%. The lowest results for the proportion of normal spermatozoa morphologically registered in the men's group over 40 years (8.6%).

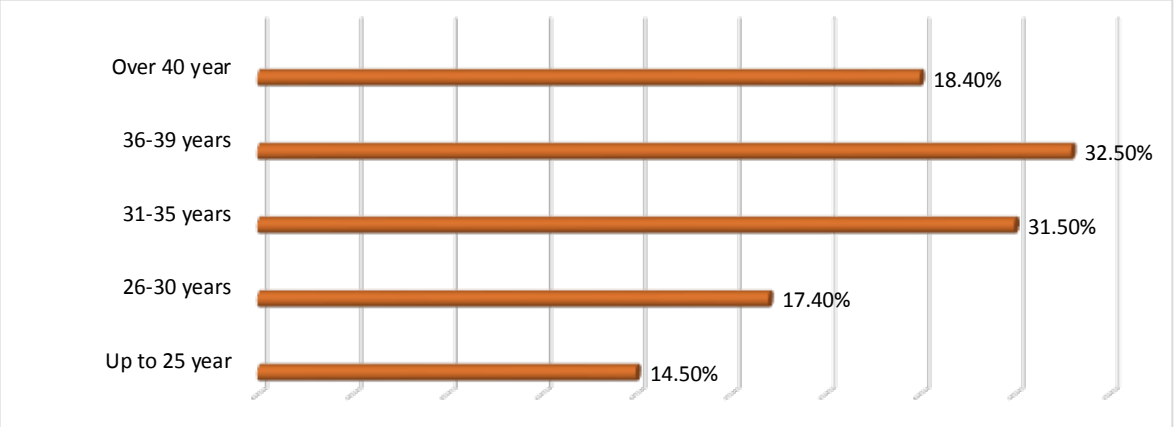

Figure 2 – The share of active motion spermatozoids in ejaculations of different ages of men

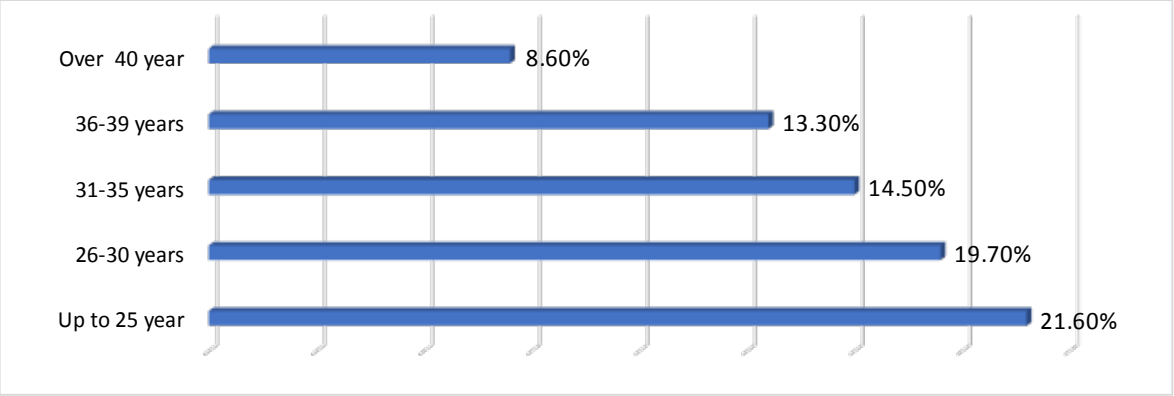

Figure 3 – Morphologically-moderate spermatozoa in ejaculations of different ages of men

In determining the morphologically-significant share of spermatozoa in the normal ejaculate, the group under 25 was the most promising group (21.6%) (Figure 3).

Comparably, the results of the group of young people between the ages of 26 and 30 were fairly high. The share of normal spermatozoa in the ejaculate in this group was 18.7%. Converting data from our study on the age-related male sperm relationships, genetic causes are the main cause of male relative malformation between 25 and 26-30 years on spermatozoa concentrations and active motion spermatozoa fraction.

The data presented show a sharp decline in the process of spermatogenic activity of male registered in the world since the second half of the twentieth century. This phenomenon is a manifestation of the daily environmental impact of the harmful factors on the human body. Many of the harmful factors (professional, natural, and domestic) can only cause

spermatogenesis only if it is sufficiently affected individually, but with long-term effects.

In order to effectively protect the human factor from the affect of spermatogenic activity, it is required to fully understand the mechanisms of their individual and joint harmful effects.

The fact that medical specialists and society pay serious attention to the problem of male spermatogenic activity only in the last decade may indicate a significant compensation stock of the reproductive system, which is observed only if the violations are only aggravated by serious cumulative effects.

In the third series of studies, quantitative and qualitative indicators were evaluated to assess the efficiency of the crusher. It was calculated using 3 types of cryoprotectors for the freezing of the obtained sperm. These analyzes have shown significant quantitative and qualitative results (Sperm Freeze – KITAZATO (Japan, 2012), Sperm Freezing – ORIGIO (Denmark, 2010), Sperm Freezing Medium – GLOBAL (Canada, 2008) (Figure 4).

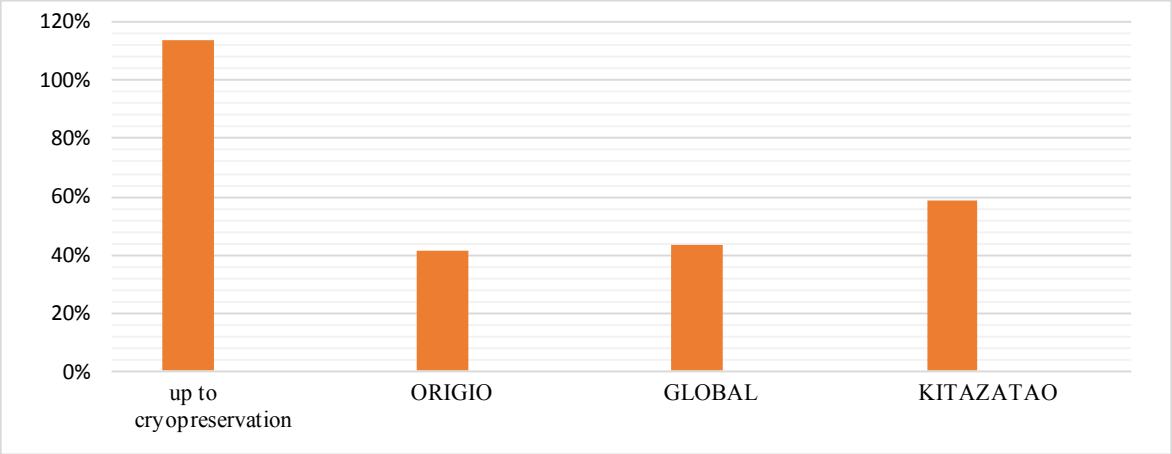

Figure 4 – Variations in quantitative indicators of spermatogenesis

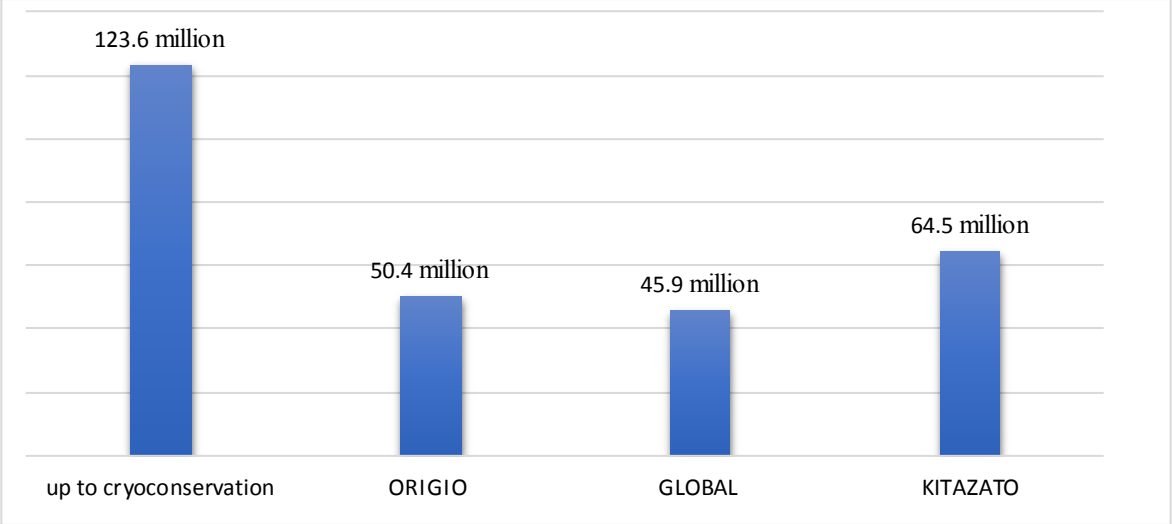

Figure 5 – Quantity of sperm in 1 mL of ejaculation and the efficacy of cryopreservation

The number of spermatozoids that are active in the total number of 60 men before cryopreservation is on the average – 114% – 123.6 million.is showed. After cryopreservation, sperm Freeze – KITAZATO – 58.8% – 64.5 million, Sperm Freezing – ORIGIO – 41.7% – 50.4 million; Sperm Freezing Medium – GLOBAL – 43.5% – 45.9 million (Figure 5).

The percentage of morphological spermatozoids in ejaculate has decreased by 73.80% compared to cryoconcentration, KITAZATO method – 54.50%, Sperm Freezing – ORIGIO-36.6%, Sperm Freezing Medium – GLOBAL method – 42.2% (Figure 6). Compared to Sperm Freeze – KITAZATO method for the freezing of sperm, 58.8% – 64.5 mL were the highest quantitative and qualitative indicators.

In addition, when the number of normal sperm motions required for fertilization is very small, their removal requires a great deal of effort and time. There is a need to keep the separated spermatozoids by cryopreservation when such risks are unlikely to occur again or physically impossible to obtain.

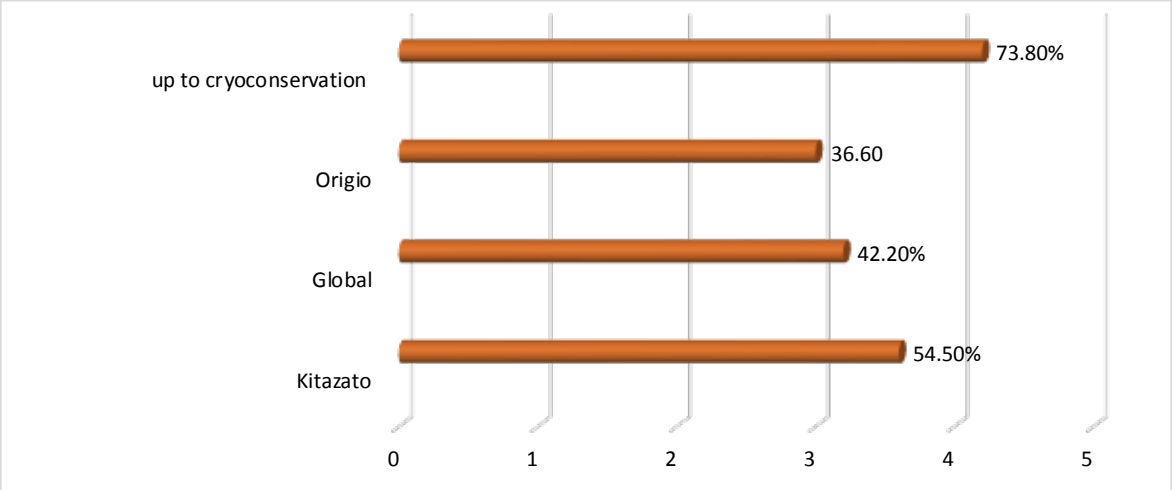

Figure 6 – Study on morphological parameters of spermatozoa ejaculate

Finally, it should be noted that the treatment of spermatozoa, which can be caused by the disorder of gonads, leads to the preservation of the ability to breed generations for patients suffering from oncological diseases. In the future, it is possible to freeze spermatozoa in patients who have been microsurgical for artificial insemination.

Conclusion

Semen or spermatozoa cryopreservation (commonly called sperm banking) can be used for sperm donation where the recipient wants the treatment in a different time or place, or as a means of preserving fertility for men undergoing vasectomy or treatments that may compromise their fertility, such as chemotherapy, radiation therapy or surgery. However, in some cases, cryopreservation might worsen the quality of the ejaculate. Thus, it is desirable to carry out freezing in test conditions to determine the need for the frozen material. One of the methodological problems of cryopreservation is poor quality of the ejaculate, i.e. when freezing spermatozoa, their activity and function deteriorate from the norm. This reduces the possibility of using them in the future for artificial insemination. Therefore, cryopreservation is possible only in specialized centers with the highest professional standards. As a result of the expertise, the group with the most promising concentration of spermatozoids was men under the age of 25. The mean concentration of spermatozoids in this group was 39.68 million times in 1 mL ejaculate. The lowest results for the proportion of normal spermatozoa morphologically registered in the men's group over 40 years (8.6%).

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