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CRYOPRESERVATION OF HUMAN SPERMATOZOA

Cryopreservation of spermatozoa plays an important role in modern assisted reproductive technologies. Today, the freezing of human sperm in liquid nitrogen is a reliable and widespread method of storage for many years. Cryopreservation of sperm is a method of storage of ejaculate, which implies its freezing and further stay in liquid nitrogen in special tanks at a temperature of -196 ° Celsius. The shelf life is not limited. Under these conditions, the biochemical processes in the cells are suspended until the time of defrosting, and their biological functions remain after defrosting. Cryopreservation of spermatozoa is a reliable protection against transmission of various diseases in their undeveloped stage. Through the use of cryopreservation in infertile couples, it is possible to use donor genetic material to find a long-awaited child. The study of cryopreservation methods has an important experimental value in the application.
Keywords: *cryopreservation, artificial insemination, spermatozoon, temperature, liquid nitrogen, freezing, auxiliary reproductive technologies.*

Cryopreservation is the freezing and storage of living biological objects with the possibility of restoring their biological functions after defrosting. Over the past decade in the development of reproductive medicine worldwide, there has been a trend towards cryopreservation of spermatozoa. The method of cryopreservation allows preserving the quality of biological material for several years, which is achieved due to careful development and study of freezing and thawing techniques. This approach provides greater controllability and effectiveness of treatment in overcoming male and female infertility. Very low temperatures are used for cryopreservation. The standard is -196 ° C. Vessels with genetic material are placed in liquid nitrogen, which provides this temperature. To use higher temperatures for storage of this biological material is inexpedient, since they are ineffective and do not allow preserving the reproductive function. One of the main problems of cryopreservation is to minimize the duration of exposure to embryos and oocytes of fundamentally harmful effects, as well as to avoid damaging the cells formed during freezing by ice crystals. Today, there are two ways of cryopreservation of embryos: slow freezing and vitrification. Slow freezing until recently was the only effective way to preserve embryos. However, using this technique, ice crystals are formed, which injure the cells. Relatively recently, an alternative method, vitrification, has become widespread. Vitrification facilitates and simplifies the process of freezing embryos. The advantage of this method is that the embryos are not damaged by ice crystals, as occurs under controlled slow cryonconservation - the liquid contained in the cells of embryos under the influence of special substances is transferred to the vitreous state [1].

The researchers found that with superfast cooling of small amounts of sperm (direct immersion in liquid nitrogen), sperm die. Exception was represented only by some samples of sperm of stallions, and also of a person. Freezing rabbit sperm in thin-walled (10 μm) aluminum bags, the sperm movement was restored only after rapid thawing in warm (380 ° C) water. With slow thawing in the air, all the spermatozoa invariably died. This death in this case is most easily explained from the position of the vitrification hypothesis. I.V. Smirnov and A.E. Bruenko found that with an increase in the thawing rate of frozen sperm granules (volume of the pellet 0.1 ml), the percentage of sperm resuming motion sharply increases. Such spermatozoa at the thawing temperature of 0°C were 19%, at 20 ° C - 30%, at 40°-43.5%, at 50°-50.55%, at 60°C -58%, at 70°C -65%. To eliminate the possible death of sperm from overheating, the sperm bottles, without waiting for complete thawing of the granules, were transferred from the "hot" baths into the water at a temperature of + 30 ° C. Obviously, with rapid heating it is possible to avoid recrystallization of vitrified sperms in the temperature zone, where the rate of crystallization processes is the highest. Note, by the way, that these data indicate that some cells die not in the process of freezing, but in thawing.

In experiments it was shown that in spermatozoa of the cock, partially dehydrated by adding levulose to the sperm, after quick freezing at -76 ° and thawing at a temperature of 42-45 °, mobility was restored. When samples were stored for many days or several months at -76 ° C, about 30% of the spermatozoa recovered. Out of 48 eggs that were smoked by the chickens, which were inseminated with frozen sperm, 12 appeared to have been fertilized, but the development of the embryo in them, observed with the naked eye, lasted no more than 10-15 hours. When later some researchers tried to use the method of vitrification of human sperm by Lyuyet, only in a small number of spermatozoa cooled to very low temperatures, after warming, mobility was restored. Not the best results were obtained in similar experiments with spermatozoa of other mammals.

In research papers Parks partially explained the reason for the differences in the results of previous researchers. He showed that a high percentage of human spermatozoa survive after cooling to -79 or -196 ° and live for a long time only when comparatively large samples of sperm are cooled in ampoules or in wide test tubes. Very rapid cooling of microscopically small samples in thin capillaries or in the form of a membrane has no effect. This was the first indisputable proof that rapid cooling and warming can harm spermatozoa or other mammalian cells. Spermatozoa of other mammals did not tolerate rapid cooling to very low temperatures, even in those cases where large samples of sperm were frozen. However, at that time, there was no danger in the ultra-fast cooling. On the contrary, the opinion was deeply rooted that the formation of ice crystals is the main cause of damage to living cells in the process of freezing and thawing [2-4].

At the present time, various cryopreservation protocols for human embryos have been developed at various stages of development: at the zygotic stage, pronucleus formation, during cleavage and blastocyst stage [5-7]. At present, methods of cryopreservation of cell cultures, tissues (blood, sperm), early (preimplantation) embryos have been developed and successfully used in medicine, agriculture and scientific experiment. Isolated organs do not tolerate cryopreservation, cryopreservation of whole organs is not developed, and their effectiveness is low. Cases of successful transplantation of cryopreserved organs are rare, as a rule, in such cases; it may not be about restoring the whole organ after defrosting, but about the presence of separate areas of living tissue in the defrosted organ. In other words, surviving after cryopreservation is not an organ as a whole, but tissue sites that can successfully survive after transplantation (for example, with transplantation of defrosted ovarian tissue). Cases of successful cryoconservation of warm-blooded animals (including humans) have not yet been recorded. Currently, there are no methods to ensure the survival of cryopreserved people, other mammals, and birds [8].

Cryopreservation of sperm can be used to preserve biological material without medical indications, i.e. at the request of the patient. The development of reproductive medicine in the early 90's allowed the fertilization of sperm with patients with oligozoospermia, as well as spermatozoa obtained surgically in patients with azoospermia. At the same time, the number of mobile normal spermatozoids suitable for fertilization can be extremely small, and their isolation is very laborious, which requires a lot of effort and time. If there is a high risk of repeated non-receipt of this unique genetic material due to the growing irreversible changes in the reproductive sphere of a man, or even such a forecast is seen - preservation of isolated single spermatozoa by cryopreservation is required [9].

Freezing of sperm without loss of cell quality became possible after the discovery of the cryoprotective properties of glycerol during cell freezing in 1949. Most cryoprotectants used now are based on glycerin. Their main drawback is toxicity, the potential of which largely depends on the dose, exposure and type of impact.

The use of glycerin occurring when the sperm freezes, in particular, promotes its supercooling. In this connection, doubts have arisen in the possibility of vitrifying protoplasm, as well as in the usefulness of rapid cooling and thawing of sperm. Many scientists believe that with deep

cooling of the sperm, vitrification does not occur, but fine-crystalline solidification of the protoplasm. It was also pointed out that vitrification of clean water is possible only at huge cooling rates - about 50,000 S / C, such speeds with deep cooling of biological objects are practically unattainable.

As a rule, cryopreservation is carried out at a temperature of -196 ° C, placing capsules with biological objects in liquid nitrogen. Less often temperatures are used (from -180 ° C to -130 ° C), which create electrified freezers, but this temperature regime is less reliable and not suitable for all objects. Using temperatures above -130 ° C is ineffective and is rarely used (for example, storage on dry ice at -79 ° C). Preservation of living objects at temperatures around zero degrees is traditionally not considered cryopreservation. The use of low temperatures ensures the stopping of biochemical processes in cells, including the stopping of metabolism and energy with the external environment, thanks to this, living objects can be preserved for as long as desired [10].

Cryopreservation of sperm in assisted reproductive technologies (ART) is applied in the following cases:

- to create a donor sperm bank - in situations where a man can not attend the day of a puncture of his wife's follicles and take the sperm on the same day.
- before the beginning of treatment with the use of drugs and technologies that may lead to a deterioration or total absence of fertilizing capacity of sperm (oncological diseases, hepatitis C, surgical interventions such as orchiectomy, vasectomy, etc.).
- prior to the commencement of official business trips, during which the influence of unfavorable environmental factors on the organism as a whole or organs (primarily gonads) involved in spermatogenesis (cosmonauts, accident liquidators at nuclear power plants, military, etc.) is not excluded.
- if necessary, the concentration and accumulation of spermatozoa with insufficient amount in the ejaculate[11].

Cryopreservation of sperm does not affect the genetic information and qualitative characteristics of spermatozoa that have successfully undergone frost and defrost. But some of the sex cells die during thawing. Survival by different data from 60 to 75%, which is enough for in vitro fertilization and artificial insemination. There are no data on the reduction in the frequency of fertilization and the successful development of pregnancy when using spermatozoa in IVF programs successfully transferred defrost. The properties of frozen sperms are retained for 3 years with proper storage. After this period, she begins to lose her qualities. But there are cases of successful application of thawed sperm in IVF programs after 13 and 21 years after cryopreservation. Technologies of freezing sperm allow to preserve the functions and morphological structure of male gametes. There is evidence of obtaining viable gametes after defrosting the sperm frozen 28 years ago.

As a rule, "strong" and genetically valuable spermatozoa are defrosted well. Different laboratories of reproductive centers conduct defrosting of sperm using different thawing techniques:

• at room temperature;
• in the freezer with the reverse freezing program;
• in a water bath heated to 37 ° C.

All of the above defrosting methods are designed to ensure that the defrosting rate corresponds to the freezing rate. Cryosolomine is removed from the Dewar vessel and is thawed in a tripod by one of the methods listed. After that, immediately wash out with special solutions to prevent the toxic effect of glycerin and assess the quality of thawed spermatozoa [12].

The main factors, except for a sharp decrease in the metabolism, to prevent the preservation of biological objects during freezing:

1. The formation of ice crystals inside cells is the most important damaging factor in cryopreservation. Water has the property of expanding with freezing: the formation of ice crystals, their volume will be greater than that of the original liquid. In this regard, the formed ice irreversibly damage the cellular structures, and the cell will die. Even small ice crystals irreversibly damage intracellular structures (nucleus, mitochondria, Golgi apparatus, endoplasmic reticulum), without integrity of which, even if the cytoplasmic membrane of the cell itself is preserved, its vital activity is impossible. The greatest probability of the formation of crystals inside cells arises from their uncontrolled freezing.
2. The formation of ice crystals outside the cells also leads to cell death due to dehydration. In the process of freezing, ice crystals are formed, and due to this, the amount of free water that is able to interact with solution electrolytes decreases. This leads to an increase in the concentration of dissolved substances in the extracellular space (the solution becomes hypertonic with respect to the intracellular environment), as well as changes in pH. Accordingly, the osmotic pressure increases, and the flow of water leaving the cell starts to increase, which leads to a decrease in the amount of water inside the cell and, as a consequence, to a breakdown in the structure of proteins. The lower the temperature, the more water outside the cell turns into ice, which means less free water remains outside the cells, and the more cells dehydrate, the more damaged their structures, and the less chance remains to restore their functionality after thawing. The formation of extracellular ice is most intense at a very slow freezing rate [13].
3. Overheating of cells at the time of the water / ice phase transition - paradoxically, in the process of freezing, cells can die from overheating. In the phase transition of extracellular water from the liquid state to the ice, which has a much lower entropy (solid) than the liquid, excessive heat is generated, which can lead to irreversible cell damage. To prevent cell death from overheating, they are very quickly cooled near the phase transition point - at the temperature of the cooled liquid equal to the crystallization start temperature of the solution (siding), triggering the formation of ice crystals.
4. Phase transitions in the lipid bilayer membranes. In the process of slow cooling at a temperature of about 0 ° C, there are strong changes in the structure of the plasma membrane-its fluidity sharply decreases and viscosity increases and, as a consequence, the permeability of the membrane for ions changes, which leads to a pH shift. The work of Na + channels stops, and this leads to the accumulation of Na + ions inside the cell, and, together with them, water. As a result, the cell strongly swells, which can bring to its lysis.
5. Cold shock - until the end unexplained phenomenon of sudden death of cells at low temperatures [14].

To prevent the formation of ice crystals that are destructive to cells, a variety of substances with relatively low freezing temperatures, cryoprotectors, are used. Cryoprotectors are substances that protect living objects from the damaging effect of freezing. Cryoprotectors are used in cryopreservation - low-temperature storage of living objects (in other words, when freezing cell cultures, blood, sperm, embryos, isolated organs and biological objects entirely). Freezing on living objects is affected by two damaging factors: the formation of intracellular ice and dehydration. The placement of living objects in solutions of cryoprotectants and freezing in these solutions reduces or eliminates the complete formation of intracellular ice and dehydration.

There are a large number of substances that possess cryoprotective properties, but in medical and laboratory practice no more than a dozen compounds are used, which will be listed below. There are cryoprotectors of two types: penetrating and non-penetrating. Penetrating cryoprotectants penetrating the cell. Penetrating cryoprotectants prevent the formation of ice crystals due to the formation of hydrogen bonds with water molecules. The most common penetrating cryoprotectants: glycerin, propylene glycol, ethylene glycol, dimethylsulfoxide.

1. Penetrating cryoprotectants are able to enter the cell and prevent the formation of ice crystals due to the formation of hydrogen bonds with water molecules. They themselves replace water, which prevents the cryodestruction of biologically important macromolecules, and bind some amount of free water (which reduces the overall dehydration of cells). In addition, penetrating cryoprotectants form hydrogen bonds with the macromolecules of the cell, which stabilizes their structure [15].
2. Cryoprotectants that do not penetrate into cells belong to the non-penetrating. The principle of the action of non-penetrating cryoprotectants is not fully understood. It is probably twofold: a decrease in the growth rate of crystals and protection of the cell from osmotic changes. Non-penetrating cryoprotectants include two groups of substances: oligosaccharides (sucrose and trehalose are most often used) and high-molecular compounds (ficoll, albumin, polyvinylpyrrolidone are most often used). The use of non-penetrating

cryoprotectants in the absence of penetrating ineffective, that is, non-penetrating cryoprotectants are additional components in solutions of penetrating cryoprotectants.
After thawing, living objects must be freed from cryoprotectants. The protective mechanism of the cryoprotector on the cell is based on such effects:
• the ability to penetrate the cell;
• the ability to dehydrate (displacement of water molecules, which leads to a decrease in crystal formations, damaging organelles and the cell wall);
• the ability to reduce the concentration of electrolytes can reduce the osmotic pressure in the cell;
• interaction with phospholipids of the cell wall leads to a decrease in the freezing point, and this protects the cell membrane.
Cryopreservation of sperm is a method worthy of respect, allowing to bypass many underwater stones of infertility. Scientific work to improve the method continues. In addition, work is underway to identify and develop alternative methods for the long-term storage of spermatozoa.

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АДАМ СПЕРМАТОЗОИДТАРЫНЫҢ КРИОКОНСЕРВАЦИЯСЫ

Түйін: Қазіргі уақытта сұйық азоттағы адамның ұрығын мұздату көптеген жылдар бойы сенімді әрі кеңінен таралған сақтау әдісі болып табылады. Криоконсервация әдістерін және оларды төмен температураның және судың мазмұнының қатуынан туындаған зақымдануды қолдану тәжірибеде маңызды эксперименталды маңызға ие. Дегенмен, бүгінгі таңда, әлемнің көптеген елдерінде сұйық азот температурасында сперматозоидтарды сақтау кейбір кемшіліктерге ие, бұл станцияларды және сұйық азотпен жасанды ұрықтандыру станцияларын үздіксіз жеткізу қажеттілігін, криогенді жабдықтардың жоғары құны, криоконсервация процесінде өлімнің сперматозоидтарға дейін қажеттілігін көрсетеді.

Түйінді сөздер: криоконсервация, сперма, жасанды ұрықтандру, сперматозоид, температура, сұйық азот, мұздату, қоымша репродуктивті технология

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КРИОКОНСЕРВАЦИЯ СПЕРМАТОЗОИДОВ ЧЕЛОВЕКА

Резюме: Криоконсервация сперматозоидовов играет важную роль в современных вспомогательных репродуктивных технологиях. На сегодняшний день замораживание в жидком азоте спермы человека является надежным и широко распространенным способом хранения в течение многих лет. Криоконсервация спермы это метод хранения эякулята, который подразумевает его замораживание и дальнейшее пребывание в жидком азоте в специальных резервуарах при температуре -196° по Цельсию. Срок хранения при этом не ограничен. В этих условиях биохимические процессы в клетках приостанавливаются до момента размораживания и при этом их биологические функции сохраняются после размораживания. Криоконсервация сперматозоидов является надежной защитой от передачи различных заболеваний в их непроявленной стадии. Благодаря использованию криоконсервации у бесплодных пар появляется возможность воспользоваться донорским генетическим материалом, чтобы обрести долгожданного ребенка. Изучение методов криоконсервации имеет важное экспериментальное значение в применение.
Ключевые слова: криоконсервация, исскуственное осеменение, сперматозоид, температура, жидкий азот, замораживание. вспомогательные репродуктивные технологии.