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Recent updates of sperm cryopreservation technique: a literature review

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ABSTRACT

Background: Nowadays, the decline in male fertility is a significant concern due to the mechanical, systems disturbances, genetic, diet, lifestyle, and environment. Currently, no strategies for medical protection of the germinal epithelium are available. Sperm cryopreservation technique has been established to overcome those issues. Accordingly, this study aims to informed the recent updates of sperm cryopreservation technique from kinds of literature.

Methods: A review of relevant literature was performed to elaborate the recent updates in the sperm cryopreservation technique. A total of 45 qualified published literature of all years until 2019 were collected from several electronic database and manual search and included in this review.

Results: According to the literature, the first record of sperm cryopreservation was made by Lazzaro Spallanzani in 1776. He used to snow during winter and observed that the motility of human spermatozoa could be preserved after freezing and thawing. Sperm cryopreservation programs provide temporarily or permanently infertile men an opportunity to seek paternity. *Sperm cryopreservation* is the most effective and the most strongly recommended option for fertility preservation *prior to* any treatment or *occupational gonadotoxic exposures*. Sperm cryopreservation should be discussed and offered prior to therapy for malignant diseases. Freezing sperm before the initiation of treatment provides patients with future fertility potential.

Conclusion: Sperm cryopreservation could be offered to the patient before the vasectomy procedure in order to prevent reversal vasectomy or surgical sperm retrieval in the future.

Keywords: Updates, Sperm, Cryopreservation Technique, Literature Review
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INTRODUCTION

Nowadays, the decline in male fertility is alarming.[1] It is caused by several factors such as mechanical, systems disturbances, genetic, diet, lifestyle, and environment.[2] Anticancer treatments (i.e. chemotherapy and radiotherapy) can damage the germinal epithelium in men resulting in oligozoospermia or azoospermia. Currently, no strategies for medical protection of the germinal epithelium are available.[3] Men who work in industries are exposed to radiation, toxins or other pollutants and might get lower sperm quality.[4] Some couple who have no fertility disturbance, but living apart due to many reasons. Some couple does a family planning program. Vasectomy could be chosen by and make them be an "iatrogenic" obstructive azoospermia.

Interestingly, some improvement in oncology treatment may cause an increase in survival rate. Some of these survival men may have a will to have an offspring in the future.[5] Surgical reconstruction have been performed to overcome obstructive azoospermia. However, in some cases, these procedures might not be able to reverse fertility due to obstruction. It is possible to preserve male fertility if we could keep their sperm before anticancer administration, any industrial and environment gonadotoxic exposure, or vasectomy.

The demand for Assisted reproductive technology (ART) is growing now. It is due to the availability of sperm is a compulsion for this procedure. Sperm cryopreservation before anticancer, gonadotoxic agent or male contraceptive procedure is the best choice to ensure the sperm availability for ART until now. This procedure also could provide the sperm availability for the couple who want to have a child but had trouble to have regular (or during the periovulation period) sexual intercourse. Intraoperative cryopreservation is recommended for complex reconstructive cases with lower patency rates. Based on those mentioned above, this literature review aims to discuss further the recent updates of sperm cryopreservation technique.

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HISTORY OF SPERM CRYOPRESERVATION

Lazzaro Spallanzani made the first record of sperm cryopreservation in 1776. He used snow during winter and observed that the motility of human spermatozoa could be preserved after freezing and thawing.[6] Mantegazza observed in 1886 that human sperm survived to cool to −17°C for more than 4 days. He speculated that in the future, frozen semen might be used in animal husbandry and even proposed that a man dying on the battlefield might, by his wife, beget a legitimate child after his own death.[7]

Sixty years later, Polge discovered that glycerol was an effective cryoprotective agent (CPA) for freezing sperm. Since then, glycerol has been the primary CPA used for freezing sperm from most animal species, including humans.[8] The discovery that glycerol was an effective CPA quickly resulted from the development of cryopreservation techniques that maintained fertilizing ability. In 1953, Bunge and Sherman reported the first pregnancy resulting from cryopreserved human sperm, which had been frozen using glycerol.[8]

Until 1963, human semen was preserved by the "dry-ice" method. It was reported that only 50% of sperm could survive in term of motility after hours to weeks storage. Sherman said that storing human sperm in liquid nitrogen (-196°C) was superior to the previous method following three years of successful application. It was reported that post-thaw survival with this nitrogen-vapour method is superior to original "dry-ice" (-75°C) and expensive automatic freezing methods. He noted no loss due to storage after more than 4 years.[9] Before 1964, all pregnancies were produced from the short-term storage of sperm. However, Perloff et al. reported pregnancies from insemination with frozen-thawed sperm stored for one to 5.5 months using glycerol and liquid nitrogen.[10]

The introduction of in vitro fertilization and intracytoplasmic sperm injection (ICSI) overcome severe male infertility problem.[11,12] Since then, the use of cryopreservation of ejaculated, epididymal, and testicular sperm has been grown rapidly. The first case of a successful pregnancy with in vitro fertilization (IVF) using cryopreserved sperm from a man with a testicular malignancy was reported in 1985. Successful birth using epididymal sperm had been reported even it was frozen 24 hours after conventional oocyte insemination.[11] Some case reports of successful ICSI with frozen-thawed testicular motile sperm have been published in 1996.[12–15]

INDICATION OF SPERM CRYOPRESERVATION

Sperm cryopreservation programs provide temporarily or permanently infertile men an opportunity to seek paternity.[13-15] Sperm cryopreservation is the most effective and the most strongly recommended option for fertility preservation prior to any treatment or occupational gonadotoxic exposures.[9,10] Sperm cryopreservation should be discussed and offered prior to therapy for malignant diseases. Freezing sperm before the initiation of treatment provides patients with future fertility potential. Sperm cryopreservation before vasectomy procedure should be provided in order to prevent reversal vasectomy or surgical sperm retrieval in the future.

Sperm cryopreservation before ART could ensure the availability of sperm at the time of ICSI. Supernumerary of sperm after surgical sperm retrieval could be stored and used for the next cycle IVF.[14] In order to prevent recurrent surgical fertilization due to obstruction, sperm freezing should be carried out. The several methods of cryopreservation are mentioned to the following paragraphs.

FREEZING METHOD

Slow Freezing

Slow freezing is one of the most commonly used protocols for human sperm cryopreservation. The slow freezing technique proposed by Behrman and Sawada consists of progressive sperm cooling throughout 2–4 h in two or three steps.[16,17]

The manual method is performed by simultaneously decreasing the temperature of the semen while adding a cryoprotectant in a stepwise manner. It has been shown that the optimal initial cooling rate of the specimen from room temperature to 4°C (or 5°C) is 0.5–1°C/min. The sample is then frozen in liquid nitrogen vapours (LNV) for 20 minutes to decrease the temperature from 4°C (or 5°C) to – 80°C at a rate of 1–10°C/min. The specimen is then plunged into liquid nitrogen at −196°C.[17,18]

During cooling at LNV, freezing devices containing sperm samples to be cryopreserved must be arranged to minimize temperature variations along with the cryo-storage device. In slow freezing, freezing rates are controlled manually by the operator, so variations in the freezing rate are more likely to occur.[18] When the freezing rate is above the optimal, there is a more significant mechanical injury because the ice-crystal formation is promoted, whereas a below-optimal freezing rate promotes osmotic shock-induced damage.[18]

Programmable Freezing

Programmable freezing provides a stricter control of freezing rates through the use of automated programmable LN freezers.[6] Therefore, for programmable sperm freezing, sperm samples mixed with cryoprotectants are arranged on a plate before the freezing rate is selected. Samples are frozen first using a freezing rate of -1.5°C/min, decreasing the temperature from 20 to -80°C.[4] Next, the freezing rate is increased to -6 °C/min. Once the temperature decrease is finished, samples will be submerged in LN (-196 °C).[4]

The usefulness is limited to those circumstances when a high number of samples are required to be cryopreserved at the same time. Furthermore, in some conditions, programmable sperm freezing has been described as a less efficient process because of latent heat leading to delays in freezing rates, thus being detrimental for spermatozoa.[5]

Rapid Freezing

In the rapid freezing technique, spermatozoa are mixed with the cryoprotectant and the suspension is loaded into a cryo-straw or cryovial. After that, it exposed to an LNV phase for 10 to 20 minutes before being plunged into liquid nitrogen.[6,17]

The freezing rate during this protocol depends on the period of the samples incubated as well as the distance to LNV. The strengths of rapid freezing are the minimizing of procedural time, and it does not require high-technology devices.[18]

Vitrification of Human Spermatozoa

Sperm vitrification is characterized by the formation of solid glass-like structures preventing ice crystal formation. Sperm vitrification is a non-equilibrium cryopreservation procedure which achieves ultra-rapid freezing rates employing submerging samples in LN (-196 °C), thus bypassing ice-crystal formation.[18] The achievement of such ultra-rapid freezing rates requires a high concentration of cryoprotectants; hence, osmotic shock due to high levels of cryoprotectants is the leading cause of cryopreservation-associated damage during sperm vitrification.[18]

Before sperm vitrification, it is mandatory to perform a sperm preparation to remove seminal plasma.[18] Unfortunately, one of the major limiting factors for implementing sperm vitrification in the standard clinical practice is the lack of acceptable reproductive outcomes when large volumes of samples are vitrified.[18] Nevertheless, there is a growing tendency to implement and improve vitrification as a cryopreservation technique using a small amount of sperm suspension to maximize the contact surface area with liquid nitrogen.[19]

According to the results of a meta-analysis conducted by Li et al., vitrification is superior to conventional methods regarding total and progressive motility.[19,20] However, the efficacy of vitrification is influenced by using a different protocol. The results of this meta-analysis are also limited by the small number of studies of variable vitrification protocol. Further, well-conducted studies are required to confirm the efficacy of vitrification in cryopreservation of spermatozoa.[20]

FREEZE DRYING

Freeze drying sperm is based on the principle of ice sublimation. The sperm sample is first frozen to a solid ice state with the addition of suitable lyoprotectants then heated in a lyophilizer under low temperature and pressure below the critical temperature of water to allow sublimation. The advantage of freeze-dried sperm is that the sample can be easily transported over long distances, the freeze-drying procedure does not involve the use of cryoprotectant and liquid nitrogen-based storage.[21]

Many animal studies show that the freeze-dried sperm, even impaired in their viability, motility and DNA integrity, can be used for successful oocyte fertilization through ICSI.[22–25] Interestingly, the DNA integrity damage by freeze-drying in humans is lower than that of cryopreservation.[26,27]

FREEZING AFTER SPERM PREPARATION

Several studies agree to the idea of freezing prepared sperm samples, so sperm selection techniques are performed before cryopreservation to improve survival after thawing.[20-25] Performing the sperm preparation prior to sperm freezing implies both the removal of seminal plasma and the selection of a potential subpopulation of spermatozoa exhibiting better sperm parameters, such as motility and morphology.[23,24]

Swim-up (SU) and density gradient centrifugation (DGC) are the two foremost in vitro sperm preparation techniques routinely used in clinical practice to select the best subpopulation of spermatozoa and performed prior to ART purposes.[22-24] Sperm preparation before freezing might remove several compounds can be found, such as dead spermatozoa, debris, and leukocytes that induce ROS production, all of which can cause damage to sperm cells.

Esteves et al. and Petyim et al. suggested SU before freezing improve sperm motility in normozoospermic patients.[28,29] Brugnon et al. performed another sperm preparation technique, DGC and found that higher recovery rates of progressive and total motile spermatozoa were observed when the sperm selection was performed

before freezing.[30] We found that more normal morphology was recovered when sperm preparation using mini DGC were performed before freezing.[31]

DIRECT FREEZING

In this protocol, sperm preparation is performed after sperm freezing and leads to the selection of the best spermatozoa in the thawed sample. At the same time, cryoprotectant agents are also removed during sperm preparation to allow its usage in the following ART. Several studies propose performing the sperm selection after freezing as a protocol that leads to an increase in the total amount of spermatozoa with good motility.[32,33]

Sperm viability was significantly higher for samples cryopreserved using direct freezing protocol either when TEST Yolk Buffer or Sperm Freeze was used as freezing medium.[32] Freezing before sperm selection leads to higher total and progressive motility, total motile sperm count, and viability rates than when sperm selection is performed before freezing ($P < 0.005$ in all cases).[33]

Seminal plasma is an ideal medium for sperm preservation prior to capacitation. Several substances in the seminal plasma might act as protective factors against Radical Oxygen Species (ROS) release.[31-34] Antioxidant enzymatic systems such as catalase, glutathione peroxidase, and superoxide dismutase, as well as other non-enzymatic compounds like ascorbic acid, E vitamin, carotenoids, and ubiquinones, are found in the seminal plasma. Poly-unsaturated fatty acids in the seminal plasma also contribute to the antioxidant capacity of the seminal plasma and cryoresistance by increasing fluidity of sample.[34]

Moreover, animal studies also support the seminal plasma cryoprotective properties. The physical adsorption of proteins present in the seminal plasma to the sperm cell surface helps to prevent temperature shock-induced damaged.[35] Patel et al. suggested that heparin-binding proteins also have a protective function against temperature shock by preventing lipid peroxidation.[36]

Interestingly, when Total motile sperm count (TMSC) is calculated from the data derived from the studies carried out by Petyim and Esteves, it is found that TMSC is higher when sperm preparation is performed after sperm freezing. Therefore, it could be concluded that sperm preparation after sperm freezing is the protocol that leads to higher values of TMSC.

EPIDIDYMAL AND TESTICULAR SPERM CRYOPRESERVATION

Spermatozoa obtained during surgical sperm retrieval have a low number of sperm and a little percentage of progressive motility. Due to cryopreservation reduces the values of sperm motility, it would be fruitless to attempt conventional in vitro fertilization using cryopreserved epididymal or testicular spermatozoa. However, using frozen epididymal or testicular sperm for ICSI is a reliable method.[37,38]

In obstructive azoospermia cases, considering the success rate of sperm retrieved, freezing can be performed either on a diagnostic occasion or the day of oocyte retrieval. However, several considerations should be done in non-obstructive azoospermia (NOA) cases. A meta-analysis of ten studies involving 734 treatments showed a lower implantation rate of frozen-thawed testicular sperm ICSI compared with fresh sperm ICSI (relative risk: 1.75; 95% CI: 1.10-2.80).[39] In NOA with poor testicular quality, a fresh retrieval is preferably scheduled as back-up on the day of OPU.[40]

Testicular spermatozoa are preferably frozen in suspension, obtained after mechanical or enzymatic treatment procedures. Testicular tissues are minced and washed to release the sperm from seminiferous tubule. Erythrocyte lysis buffer should be used for removing erythrocyte from the sperm suspension.[41]

FREEZING A LOW NUMBER OF SPERM

Current sperm procedures used for sperm freezing/thawing lead to a decrease of more than 50% in motility and survival rate. This conventional method also usually involves dilution of the sample with cryoprotectant before loading into the cryovials or straws. There is often a loss of sample due to adherence to the vessel wall.[42] For a healthy man with the total count of sperm per ejaculate is more than a hundred million, the reduction in quality and quantity of motile sperm will not be so problematic. Several millions of motile and intact sperm following thawing will be sufficient for an ART.

However, the semen of oligoasthenteratozoospermic (OAT) men often contains a minimal number of spermatozoa, and they are always candidates for sperm freezing and fertility preservation. Little efficiency of the current method usually become a problem because of the low number and quality of sperm. Moreover, cryovials and straws are not suitable for cryopreservation of limited amounts of spermatozoa.[42] The method enabling cryopreservation of individual spermatozoa in extreme cases of oligozoospermia would tremendously benefit the patient.

During the past decade, many researchers attempted to invent new technologies in particular for freezing of individual or limited numbers of

Table 1. She several types of carrier which could be used for low number sperm cryopreservation

Carrier	Advantages	Disadvantages
Empty zona	Easy to handle the zona envelope; good sperm recovery and survival	Human or non-human biological material; Labor-intensive; Requires micromanipulation and in-house preparation of the zona envelope
Mini straws or open-pulled straws	Easy and simple technique	Not feasible for a very low volume/number of sperm
ICSI pipette	Commercially available	Fragile glass pipette
Microdroplets	Easy and simple technique	Difficult to handle and store in liquid nitrogen; Culture dishes are fragile when stored in liquid nitrogen
Volvox globator algae	Inexpensive; plentifully available	Non-human biological material; labor-intensive advance preparation of algae spheres
Alginate beads or agarose microspheres	Inert polymers used as carriers	Very labor-intensive technique
Cryotop	Commercially available	
Cryologic®	Commercially available	
SpermVD®	Commercially available	

human spermatozoa in men with severe male factor infertility.[42,43] They applied several biological and non-biological carriers including human, mouse and hamster zona pellucida, agarose and alginate microspheres, ICSI pipettes, cryoloops, mini-straws, microdroplets and many other carriers for cryopreservation of several small aliquots or even small numbers of sperm to preserve fertility especially for men with transient azoospermia. Using Cryologic®, we concluded that sperm in any amount could be preserved.[43] The several types of the carrier which could be used for low number sperm cryopreservation are depicted in Table 1.

TESTICULAR TISSUE CRYOPRESERVATION

Anticipating that new therapies will be available in the future, many centres have determined to preserve testicular tissue for young patients. They are at high risk for infertility and have no other options to maintain their fertility. Isolation of spermatogonial stem cells from cryopreserved tissues would be a suitable cell source for in vitro maturation or future autologous intratesticular transplantation after cure to re-establish spermatogenesis after cancer therapy.[44] Spermatogonial stem cell transplantation from frozen/thawed testicular cell suspensions have been reported for seven Hodgkin's disease patients in the UK, but fertility outcomes from that study have not been reported.[45]

CONCLUSION

Sperm freezing is one of fertility preservation that allows sperm storage with an unlimited period and ensures the availability of sperm for ICSI. Several sperm freezing methods are available. Sperm in any amount even single could be preserved. Testicular spermatozoa are preferably frozen in suspension, obtained after mechanical or enzymatic treatment procedures. Testicular tissue preservation is indicated for young patients who are at high risk for infertility and have no other options to preserve their fertility.

CONFLICT OF INTEREST

There is no competing interest regarding the manuscript.

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AUTHOR CONTRIBUTION

All of the authors are responsible for the study from the conceptual framework, data gathering, analysis, until reporting the results of the study through a narrative form for the literature study.

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