A review of methods for preserving male fertility

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Summary
Male infertility is responsible for 50% of men’s health problems and has always been a concern for personal and social issues. A survey of global statistics suggests an increase in infertility rate as one of the critical issues documented in studies. There are different ways of maintaining fertility in men, depending on their age. In this paper, we review the preservation methods used for fertility treatment in Iran and other countries. Available data were reviewed from Google Scholar, PubMed, Scopus, Web of Science, IranMedex, MEDLIB, IranDoc and Scientific Information Database and searched for articles published up to 2018, using the medical subject heading (MeSH) terms for cryopreservation, sperm, testicular, spermatogonia stem cell, male infertility and/or Iranian and in the world, to provide evidence from evaluation of fertility preservation the methods. Based the search strategy, 274 manuscripts were found. After reviewing the titles, abstracts and manuscripts in their entirety, 119 articles were obtained and selected according to the eligibility criteria. The 85 studies mentioned above were divided into three categories (sperm, testis, and spermatogonia stem cells (SSCs)), and methods of fertility preservation were investigated. Ways to maintain male fertility were different depending on age, and included sperm, testicular, and SSC freezing. The number of studies on testicular tissue and SSCs was low for human samples, and more studies are still needed. Sperm freezing at infertility centres is the top for male fertility preservation.

Introduction
A quarter of Iranian couples and 15% of couples in other countries are involved in primary infertility (Agarwal et al., 2015). Factors, including age, fertility status of the female partner, duration of infertility, primary and secondary infertility, and results of semen analysis, play a significant role in the prognosis of male infertility (Kumar and Singh, 2015). A male partner study includes a complete medical history and a physical examination based on the WHO standardised plan so that any infertility related to men can be detected (Bieniek et al., 2018). Chemotherapy and radiation therapy in adults and paediatric patients can cause complications related to their fertility; 30% of childhood malignancy treatments affect fertility, and the prevalence of infertility in these individuals is significant (Azizi and Ghafoori-Fard, 2017; Schuppe et al., 2017; Bieniek et al., 2018).

Sperm cryopreservation, which protects and maintains sperm cells at very low temperatures, is a potential method to solve infertility issues in men. For nearly half a century, methods for freezing sperm have been developed, and at this time, freezing is one method in assisted reproductive technology (ART) (Ferrari et al., 2016). Sperm cryopreservation is useful for further applications such as artificial insemination, infertility treatments, and sperm donation (Mocé et al., 2016). Cryopreservation of testicular tissue and SSCs is another way to maintain fertility for couples with azoospermia (Hezavehei et al., 2018). In this procedure, spermatogenesis can be stored for in vitro fertilisation (IVF)/intracytoplasmic sperm injection (ICSI) while reducing patient costs and surgery risks. Spermatogonial stem cells are also present in these tubules (Mocé et al., 2016; Robinson, 2018). These methods could also be helpful for preserving the fertility of prepubertal boys undergoing chemotherapy/radiotherapy. In summary, the purpose of this systematic review article was to investigate various freezing methods, applications, disadvantages, and advantages of freezing sperm.

Materials and methods

Search strategy and databases
A comprehensive literature review was performed to retrieve original Persian and English language papers for ‘A Systematic Review of Male Fertility Preservation Options, Benefits and Limitations’ deposited in international and Iranian databases including ProQuest, Google Scholar, PubMed, Scopus, IranMedex, and the Scientific Information Database (SID). All
relevant papers that contained selected key terms (spermatogonia stem cell, sperm, testis tissue, preservation, freezing methods, male infertility, and Iranian regional and international populations) and published up to 2018 were included. Furthermore, reference lists of the extracted articles were checked to find other useful sources. Authors of articles were contacted to ask for clarification when inadequate information was provided.

**Selection criteria**

The selection of articles occurred first through the analysis of titles and abstracts. The reviewed articles had the following characteristics: (i) papers had in the title at least a mixture of the terms outlined in the search strategy; (ii) articles were written in English or Farsi; and (iii) papers were indexed in one of the databases mentioned above. The excluded elements encompassed: (i) papers repeated in more than one database, which were then counted just once; and (ii) non-original papers such as Letters to the Editor, Brief Communications, Corrections/Editorials, and Monographs.

**Data extraction**

We read each sample item in its entirety and recorded the information on a spreadsheet that included authors, year of publication, sampling, preservation method, and significant findings. Some of the manuscripts focused on the theme of male infertility correlated to other causes that were not related to preservation and freezing methods. Due to heterogeneity in the literature, we carried out a qualitative analysis. According to the strategy adopted initially, we found 274 manuscripts and reviewed the titles, abstracts, and manuscripts it their entirety, then we selected 119 articles according to the eligibility criteria. The 85 studies were divided into three predetermined categories: (i) testis tissue cryopreservation; (ii) sperm cryopreservation; and (iii) spermatogonial stem cell cryopreservation, benefits, and limitations.

**Results**

**Male fertility preservation methods**

Fertility preservation methods can be divided into two categories according to the age range of patients. The first category includes methods for fertility preservation in adult males; these methods were relatively accessible due to the availability of testicular tissue and effective spermatozoa (Hotaling et al., 2016; Dearing et al., 2017). The second category was fertility preservation methods for children undergoing chemotherapy who are faced with a lack of spermatogonial cells and no adequate sperm. As a result, providing conditions for maturity and differentiation of these cells into spermatocytes and spermatozoa is an essential part of this process (Hotaling et al., 2016; Rahiminia et al., 2017). All approaches to fertility preservation in children and prepubertal boys face many problems including ethical, practical and scientific issues. For young men, testicular biopsy cryopreservation is the main approach to save their future fertility. However, how to use testicular tissue in the future is not clear. Conversely, there has been no credible evidence that fertility could be preserved through this approach (Anderson et al., 2015).

Fertility preservation methods for adults, protection of the gonad in situ, and sperm cryopreservation have been established for a long time. Protection of gonads in situ include shielding of the gonad from radiotherapy and replacing alkylating agents and alternative drugs such as dacarbazine. Although fertility preservation methods for adults have been performed routinely, some challenges such as high costs, cultural differences, the age of patients, and sociodemographic characteristics have reduced the rates of these procedures. (Flink et al., 2017).

Testicular germ cells in adult males using GnRH analogue treatment after conducting radiation or chemotherapy has led to an increase in the number of differentiated germ cells. (Shetty and Meistrich, 2005). Meistrich et al. (1999) suggested that 90% of people who were given GnRH after 3.5 Gy irradiation had spermatogonia, although its effect on fertility is still unknown. Jafarian et al. (2009) showed in an azoospermia mouse model that the injection of gonadotropins with oestradiol was associated with increasing return spermatogenesis.

Lack of testicular tissue differentiation and spermatogenesis in prepubertal boys is the main problem for fertility preservation approaches in young males. (Oktay et al., 2018; Walschaerts et al., 2018). Therefore, their fertility preservation should be based on one of three following options: (i) testis tissue cryopreservation and attempt to reconstitute tissue after recovery; (ii) isolation of spermatogonial cells and attempts at sperm production; and (iii) isolation of spermatogonial cells from frozen testis tissue after treatment of cancer and its refinement from cancer cells, and re-transplantation after recovery (Yumura et al., 2018; Song et al., 2019).

**Sperm cryopreservation**

Freezing sperm is the easiest type of cell cryopreservation compared with cryopreservation of embryos, eggs, and ovarian tissue, therefore frozen semen have been used in many ARTs in laboratories (Silber, 2018a). Cryopreservation of sperm currently plays a vital role in preserving the fertility of couples undergoing infertility treatment, chemotherapy or radiation therapy, or for patients with cancer (Hotaling et al., 2016; Dearing et al., 2017). There is a possibility of damage to the gonads in patients with degenerative diseases such as diabetes, multiple sclerosis or spinal cord injury, as well as men under surgery such as vasectomy, screening and quarantine of the donor sample, oligospermia and cryptozoospermia (Jang et al., 2017; Wang et al., 2018). Polge and colleagues in 1949 showed that sperm could survive at ~70°C in the presence of glycerol (Polge et al., 1949), and was the beginning of the theory of freezing sex cells and tissues (Berkovitz et al., 2018).

Some agents such as glycerol, egg yolk, and propanediol (in some other protocols) as cryoprotectants have led to decreased salt concentrations and an increase in unfrozen water fractions. Their primary function is to effectively reduce osmotic pressure in the cryopreservation procedure (Donnelly et al., 2001). Despite the variety in sperm cryopreservation methods, the basis of all procedures is as follows:

- After storing semen at room temperature for 2–3 days, sperm should be analysed for their morphology and motility. The process of sperm freezing will begin if the results of the analysis are acceptable.
- In this step, Tris yolk buffer (20% egg yolk + 12% glycerol) is added to the sample. As mentioned before, cryoprotectants (glycerol and egg yolk) reduce osmotic pressure and maintain cell membrane integrity.
- The prepared solution, citrate or a physiological salt extender, and antibiotics are vortexed and the solution divided into cryovials for storing in liquid nitrogen (~196°C) until required (Anger et al., 2003) (Figure 1).
In the freezing process, the formation of intracellular ice crystals is problematic, and the survival of frozen cells depends on the type of cell, the speed of freezing, the type of antifreeze, and the freezing method (Slabbert et al., 2015). As mentioned before, there are diverse cryoprotectants, which are divided into permeating and non-permeating agents. Although these substances prevent damage to sperm cells during freezing, high concentrations of cryoprotectants can be toxic to the cell. Different types of cryoprotective agents and their characteristics are summarised in Table 1 (Sieme et al., 2016).

The freezing mechanism is based on the fact that water in cells freezes after an appropriate heat reduction, but without any cell damage. Molecular movement is stopped, the biochemical processes of the cell are delayed or stopped effectively and, as a result, damage. Molecular movement is stopped, the biochemical processes of the cell, the speed of freezing, the type of antifreeze, and the freezing process. Some researchers have concluded that the glass freezing method protects sperm quality better than slow freezing. Studies in Iran and other countries are listed in Tables 2 and 3, and suggest that sperm freezing in men has had many successes.

Different types of freezing methods, which include slow freezing, rapid freezing, and glassy freezing (Liu et al., 2016; Le et al., 2019; Paffoni and Palini, 2019). In the slow freezing process, temperature decreases, but in the rapid cell method, a sample is placed in the liquid nitrogen level for a period of 15 to 30 min, then transferred to a liquid nitrogen reservoir (Agarwal and Tvrda, 2017). Ice crystals are formed in both these procedures (Agarwal and Tvrda, 2017), therefore many studies have been performed to reduce the time taken and to eliminate ice crystals and the expensive equipment needed in the slow freezing method (Pradiee et al., 2017). One way to avoid sperm damage is to use the glassy method or vitrification. In this method, samples with very high cooling velocities (720,000 km/min) and a short time scan (5–8 s) are immersed in liquid nitrogen. (Yavin and Arav, 2007; Ali Mohamed, 2015; Pradiee et al., 2017). Rall and Fahy (1985) developed an ice freezing method for the embryo with no ice crystal formation. The basis of freezing was the lack of ice crystal formation due to use of high antifreeze concentrations and high cooling rates. Isachenko et al. (2003) reported sperm glass freezing through cryo-loops without using antifreeze. The high concentrations of cryoprotectant and antifreeze agents are toxic for spermatozoa, although these can be applied for embryos and oocytes. This method increased survival and sperm motility compared with conventional freezing methods due to its high speed of sample cooling and non-use of antifreeze (Isachenko et al., 2003). Among other cryoprotectants, sugars and polysaccharides have a high glass transition temperature compared with glycerol, allowing storage at higher subzero temperatures (Oldenhof et al., 2013).

Freezing in the steam and melting phase not only causes damage to the acrosome but also causes sperm death. The effects of the freezing and melting phase on spermatozoa are quite similar in fertile and oligospermia individuals (Robinson et al., 2018). Consuegra et al. (2018) showed that the presence of an osmotic pressure difference caused by protective materials in the freezing medium damaged the sperm plasma membrane. As the most important principle in the freezing process is to reduce damage caused by intracellular ice crystals formation and by toxic salts in the cell (Consuegra et al., 2018), cooling cells gradually and removal of cell water entirely would be essential. One point to note is that, before or during the cooling process, a suitable alternative to the water released from the cell should be used (Talwar and Ghosh, 2018).

Keros et al. (2007) concluded that dimethyl sulfoxide (DMSO) worked better to protect the sperm structure during the freezing process. Some researchers have concluded that the glass freezing method protects sperm quality better than slow freezing. Studies in Iran and other countries are listed in Tables 2 and 3, and suggest that sperm freezing in men has had many successes.

**Testis tissue cryopreservation**

Cryopreservation of testicular tissue for fertility preservation is an experimental procedure that has had increasing use, with tissues frozen for over 700 patients worldwide. Preserving testicular tissue by freezing samples before chemotherapy and re-grafting them after treatment is a proposed therapeutic approach to maintain fertility in children with cancer. In this method, the ultimate goal of treatment is essentially to prevent infertility of the cancer patient (Pukazhenthi et al., 2015; Baert et al., 2018). Schlatt et al. (2002) followed transplantation of testis tissue in mice and hamsters.
through to producing a new animal generation. Schlatt et al. (2006) performed an important human study during which an ectopic xenograft of humans to mice was placed under the skin, but no meiosis division was observed. This method has not yet succeeded with human tissue. However, in another study, adult testicle tissue was transplanted in 14 cancer patients and resulted in producing spermatogonial stem cells alive. They performed an important human study during which an ectopic transplant of frozen cells and did not report gene injury (FACS method) and injected them into the testis (Kubota et al., 2006), subject to the Cambridge Core terms of use.

Table 1. Comparison of cryoprotective agents

| Cryoprotective agents | Characteristics | Toxicity | Function | Examples |
|-----------------------|-----------------|----------|----------|----------|
| Permeating            | Cross the membrane intracellular protection Small non-ionic molecules | Yes | Modulate cellular dehydration Decrease the ice; crystal size | Glycerol; dimethyl sulfoxide (DMSO) |
| Non-permeating        | Osmotically active smaller molecules | Not | Cellular dehydration Increase the glass transition temperature | Disaccharides (sucrose, trehalose) |
| Osmotically inactive macromolecules | Not able to pass cellular membranes Increase the osmolality of the cryopreservation medium Bulking agents | Not | Cellular dehydration Increase the glass transition temperature | Polysaccharides (hydroxyethyl starch, maltodextrin) Proteins (albumin, polyvinylpyrrolidone) |

Table 2. Summary of included studies about sperm cryopreservation in IRAN

| Method       | Medium          | ‘Does the freezing-thawing procedure induce sperm DNA damage?’ | Author |
|--------------|-----------------|-----------------------------------------------------------|--------|
| Conventional | GEYC + SPF      | Sperm DNA integrity was improved in the glycerol egg yolk citrate (GEYC) group compared with the Spermfreeze® (SPF) group | Ziarati et al., 2019 |
| Conventional | Sericin         | Decreased DNA fragmentation compared with those in thaws without sericin | Aghaz et al., 2018 |
| Conventional | GEYC + SNP2018  | DNA integrity was not significantly affected by this time of sublethal stress induction | Hezavehei et al., 2018 |
| Conventional | Melatonin       | The optimal melatonin concentration (3 mM) significantly decreased the intracellular reactive oxygen species levels | Najafi et al., 2018 |
| Slow freezing | TBAP            | Showed lower sperm with damaged DNA with lower malondialdehyde (MDA) levels | Shojaeian et al., 2018 |
| Vitrification | Commercial      | Sperm in vapour was healthier in terms of DNA, chromatin and acrosome integrity; DNA, chromatin and acrosome integrity were decreased in vitrification | Rahiminia et al., 2017 |
| Vitrification | PTX             | Showed that pentoxifylline (PTX) was able to improve sperm movement without any adverse effects on sperm chromatin/DNA integrity in a vitrification programme | Nabi et al., 2017 |
| Vitrification | NGF             | DNA fragmentation, the other high doses had no significant effects | Saeednia et al., 2016 |
| Conventional | Commercial      | Melatonin exerts its cryoprotective effects on spermatozoa possibly by counteracting intracellular reactive oxygen species (ROS), and thereby reduces MDA generation | Karimfar et al., 2015 |
| Conventional | Commercial      | – | | Akhavizadeh, 2009 |
| Conventional | Commercial      | Does not improve post-thaw DNA fragmentation of sperm. | Khodayari Naeini et al., 2014 |
| Vitrification | Test yolk buffered-glycerol (TYBG) | There was no significant difference DNA integrity between two methods in presence or absence of TYBG. The very rapid freezing method in the absence of TYBG showed; DNA integrity were similar to the presence of TYBG | Davishnia et al., 2013 |
| Conventional | Trolox          | – | | Minaei et al., 2012 |

One of the most critical impediments to testicular tissue transplantation is the possibility of cancer transmission through autograft transplantation of contaminated tissues because it is possible that contamination cannot be assessed accurately with the usual diagnostic procedures. Fujita and colleagues (2000) succeeded in using cells lacking CD45 as spermatogonial stem cells of the mouse (FACS method) and injected them into the testis (Kubota et al., 2003). Zeng et al. (2006, 2007) examined spermatogenesis in pig after transplantation of frozen cells and did not report gene injury during the process of spermatogenesis. Ginsberg et al. (2014) froze testicular tissue of young cancer patients and reported that this
intervention was acceptable to the patient. In the study, the structural characteristics of the human testicular tissue after freezing were maintained as a plan, and no report was observed on the quality and capacity of spermatogenesis. Monkey testicular tissue transplantation (freshly frozen) showed differentiation up to the spermatocyte stage (Shetty et al., 2018). This method is vital for preventing male infertility following cancer, and there are many successful studies in humans and animals using this method. It was suggested that it could be used in young people to maintain fertility before puberty (Baert et al., 2018). Several studies in Iran and other countries are listed in Tables 2 and 3, respectively. Studies have shown that more research is needed on testicular tissue freezing in humans.

Spermatogonial stem cell (SSCs) cryopreservation

Freezing is the best method for the long-term maintenance and survival of SSCs. At this time, different protocols have been investigated for freezing SSCs from pigs, horses, rodents, cattle, and humans (Silber, 2018b). SSCs are frozen after isolation from the testes and kept until the end of cancer treatment, then thawed and returned to the testicular tissue of the individual. The SSC transplantation history dates back to 1994 when Brinster and Avarbock succeeded in returning fertility to the mouse through SSC transplantation that produced effective spermatozoa (Brinster and Avarbock, 1994).

The rate of survival and proliferation of these cells has been evaluated after freezing by mouse grafting and, to some extent, the desired results have been obtained. Usually, in SSCs transplantation studies for tracking transplanted cells, genetic markers or proteins are used in which their gene is introduced into the germ cells of the donor. Brinster and Avarbock (1994) showed that germ cells can be frozen for a long time before meiosis. In a study by Nagano et al. (1998), before SCC transplantation, germ cells were cultured in vitro, then live cells were transplanted. Results showed that the grafted cells survived for a long time. Another study in 2003 reported the results of this type of transplantation in mice (Kanatsu-Shinohara et al., 2003). SSCs produced sperm in different animal species such as rats, hamsters, goats, and cows when this transplantation was performed between two close species. Using this method, part of meiosis in spermatogenesis would be impaired (Kubota and Brinster, 2018).

In recent decades, many studies have been performed on spermatogonial stem cells of various types and under various conditions of freezing. In a study conducted by Mirzapour et al. (2013), spermatogonial stem cells obtained from

### Table 3. Summary of included studies about sperm cryopreservation in other country

| Method       | Medium            | 'Does the freezing-thawing procedure induce sperm DNA damage?'                                           | Author                        |
|--------------|-------------------|--------------------------------------------------------------------------------------------------------|-------------------------------|
| Conventional Vitrification | Glycerol Commercial | In vitrified samples DNA fragmentation was reduced approximately one-third on average compared with slow freezing | Aizpurua et al., 2017         |
| Conventional Vitrification | Commercial | DNA integrity were reduced significantly by both methods                                              | Tongdee et al., 2015/Thailand |
| Conventional Vitrification | Commercial HTM | None                                                                                                   | Mohamed and Mohamed, 2015/Germany |
| Conventional Vitrification | G-IVF + Sucrose | No differences were found in DNA stability                                                             | Zhu et al., 2014/China        |
| Conventional Vitrification | Commercial HAS/glycerol | DNA integrity was unaffected by the cryopreservation method or presence of cryoprotectants            | Isachenko et al., 2004/Germany |
| Conventional Vitrification | Commercial Commercial | Significantly fewer sperm with DNA damage                                                               | Satirapod et al., 2012/Thailand |
| Conventional Vitrification | Commercial HAS/glycerol | Sperm DNA integrity in the cryopreserved groups was not affected by the freezing/thawing procedure, as evidenced by no significant change in the percentage of sperm head DNA | Vutyavanich et al., 2010/Thailand |
| Conventional Vitrification | TYB-buffered TYB-buffered | DNA damage after two methods was not significantly different                                             | Chang et al., 2008/Korea      |
| Conventional Vitrification | Egg yolk Free | None                                                                                                   | Nawroth et al., 2002/Italy    |
| Conventional Vitrification | Glycerol + HAS Glycerol + HAS | None                                                                                                    | Saritha and Bongso, 2001/Singapore |
| Vitrification | Cryomedium | None                                                                                                   | Raad et al., 2018/Lebanon     |
| Vitrification | GEYC | None                                                                                                   | Zhang et al., 2012/China      |
| Slow freezing | Commercial | Sperm ultra-rapid freezing may be an alternative to slow freezing with better recovery results and less apparent DNA damage | Riva et al., 2018/Argentina    |
| Slow freezing | Freezing in liquid nitrogen vapour | Cryopreservation of testicular sperm does not increase baseline levels of DNA damage                  | Steele et al., 2000/Northern Ireland |
| Conventional Freezing Vitrification | TYB HSA | Lower percentages of DNA fragmentation were observed when comparing cryo-protectant-free vitrification to conventional cryopreservation | Slabbert et al., 2015/South Africa |
azoospermia patients were frozen and thawed using DMSO, and the cells showed the ability to form colonies. A study by Kanatsu-Shinohara et al. (2003) showed that 30% of boys who survived cancer were azoospermia at puberty (Shinohara et al., 2002). Ogawa et al. (2000) performed germ cell freezing using DMSO and reported a 70% survival of thawed cells after freezing. There have been relatively limited studies on freezing of SSCs, some of which are listed in Table 4.

Discussion

Based on this review, it can be acknowledged that sperm cryopreservation is the most widely used method for maintaining fertility in different parts of the world, including Iran. In summary, the applications of frozen sperm are:

(i) preserving reproductive power throughout an individual’s life;
(ii) sperm storage and maintenance before genital surgery, which disrupts the ability to ejaculate and changes the production process of the sperm;
(iii) fertility guarantee by storing and sustaining sperm for men undergoing vasectomy (Abram McBride and Lipshultz, 2018; Gupta et al., 2018);
(iv) sperm storage and maintenance for men who wish to have their sperm available to their spouse in their absence;
(v) for men who will be exposed to unique treatments (chemotherapy, radiotherapy, etc.);
(vi) a person with reduced sperm (Clarke, 2018; Thompson et al., 2016);
(vii) a person who is not able to attend an IVF sampling day;
(viii) storing and maintaining sperm for men who are exposed to radioactive substances, X-rays, toxins, and chemicals harmful to a reproductive system;
(ix) storing and maintaining sperm for the purpose of donation (Silber, 2018a).

Currently, despite the use of modern protocols for freezing, the quantitative and qualitative parameters of live sperms after thawing have not been satisfactorily compared with the sample before freezing (Gupta et al., 2018). One of the problems in the process of sperm freezing is that there is still no medium that completely prevents sperm damage during the freezing process, so the fertility rate of frozen sperm is lower than that of the fresh sample (Di Santo et al., 2012).

Cryopreservation of testicular tissue is a novel way to maintain its long-term future. Inside the body, the environment after cancer treatment can be of high risk for return of cancer cells. In addition, the internal body environment after cancer treatment with chemotherapy medications does not possess the necessary conditions for spermatogenesis (Romero et al., 1996). Spermatogenesis in the laboratory and outside the body through testicular tissue culture can be an excellent alternative (Dohle, 2010; Curaba et al., 2011). Based on these studies, more research is needed to preserve SSCs and human testis.

In conclusion, according to the results of human studies and recent advances in freezing, it seems that sperm freezing is a good way to maintain fertility. But preserving the fertility of children with cancer who do not have sperm or mature tissue should be through testes tissue and SSC preservation methods.

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Ethics standards. This is a systematic review article that examines past studies and the references used in the text.

Consent for publication. Not applicable

Data availability. All data searched in this study are included in this publication.

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Table 4. Summary of included studies about testis tissue and SSCs cryopreservation

| Type          | Sperm medium                  | Author                          |
|---------------|--------------------------------|---------------------------------|
| Testis tissue | DMSO                           | Rezaei/Iran (Topraggaleh et al., 2019) |
| Testis tissue | DMSO                           | Sadri-Ardekani et al., 2016/Netherlands |
| Testis tissue | DMSO + sucrose                 | Curaba et al., 2011/Belgium     |
| Testis tissue | DMSO + HAS                     | Keros et al., 2005/Sweden       |
| Testis tissue | DMSO + PrOH + glycerol         | Keros et al., 2007/Sweden       |
| Testis tissue | Ethylene glycol + sucrose      | Kwist et al., 2006/Denmark      |
| Testis tissue | DMSO + sucrose                 | Wyns et al., 2008/Belgium       |
| Testis tissue | DMSO                           | Ginsberg et al., 2014/Philadelphia |
| Testis tissue | DMSO                           | Yango et al., 2014/California   |
| SSCs          | DMSO + FCS                      | Mirzapour et al., 2013/Iran     |
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