

A VIEW OF PAST, CURRENT, AND FUTURE STUDIES IN CRYOBIOLOGY AND FERTILITY PRESERVATION

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Abstract

For many years, cryopreservation has been used to preserve living biological specimens. The increased risks to human fertility from diseases like cancer and their treatment protocols, such as radiation and chemotherapy, as well as the general lifestyle trend toward later marriages, have led to its expansion into the field of fertility preservation. New scientific methods, like cryostorage, which involves storing live cells and tissues at low temperatures and reviving them when needed, have greatly benefited the use of assisted reproductive techniques (ART) to preserve fertility. In order to create a future roadmap for this field, this review focuses on "cryopreservation science monitoring in reproductive biomedicine" and assesses knowledge, trends, driving forces, impetus, and emerging technologies. The importance of strategically planning cryobiology research to support its wider use in therapeutics in the future is highlighted by our analysis of the field. Recognizing this need, the Royan Institute in Tehran, Iran, has created a strategic plan to conduct interdisciplinary research on the use of cryobiology, including cryobioengineering, in disease mitigation. We anticipate that this study will contribute to an increase in the quantity and quality of expert knowledge and public discussion regarding cryopreservation's role in fertility preservation within ART.

Keywords: cryobiology; cryopreservation; fertility preservation; reproduction

INTRODUCTION

Through an analysis of existing information and an evaluation of the factors behind cutting-edge new technologies, this research aims to clarify the alternatives available today and detail potential developments for human fertility preservation. Both medical and non-medical factors make advancements in human fertility preservation crucial.

Iran has a far lower cancer incidence rate than many other nations. Iran's cancer incidence rate is 150 per 100,000 people, whereas the average yearly incidence of cancer worldwide (excluding skin cancer) is 182 per 100,000 people, according to the global reference study Cancer Registration in Five Continents (1). Furthermore, the average incidence of cancer in the United States is 318 per 100,000 and in European nations it is 267 per 100,000, indicating a lower incidence of cancer in Iran than in the United States and Europe. But according to data from the Iranian Cancer Association, cancer rates are alarmingly rising each. Women are four times more likely than the global average to develop cancer. Three years ago, there were twice as many Iranian women under the age of thirty who had cancer than non-Iranian women. In addition to the effects of increasing cancer risk factors, population growth, and ageing on cancer incidence in developing nations (which account for 82% of the world's population), Iran's cancer rate appears to be higher than it was prior to the establishment of an accurate and regular cancer incidence system following the creation of the National Cancer Registration Network (2). For women of reproductive age, breast cancer is currently the most common type of cancer. It shows that women under 40 years of age account for 15% of cases of this form of cancer. Another prevalent cancer form among women of reproductive age is cervical cancer. As a result, medical researchers have traditionally focused on cancer, and significant efforts have been made to treat the illness. The number of cancer survivors has grown due to recent advancements in cancer detection and treatment throughout childhood and adolescence. However, many individuals suffer from lifelong infertility as a result of therapies like chemotherapy and radiation. As of right now, nothing is known about their odds of becoming pregnant following cancer therapy (3).

Patients with various cancer types, such as muscle-skeletal cancers like Ewing's sarcoma, osteosarcoma, haematopoietic cancers like leukaemia and lymphomas, neuroblastomas, and Wilms tumours, as well as those with systemic diseases like systemic lupus erythematosus, acute glomerulonephritis, and behcet's disease, run the risk of losing their fertility during treatment (4).

Nowadays, people with premature ovarian failure (POF) and a range of cancers and other illnesses are thought to need to preserve their fertility. Globally, special initiatives have been established to protect fertility in

individuals receiving radiation or chemotherapy (5) (Figure 1).

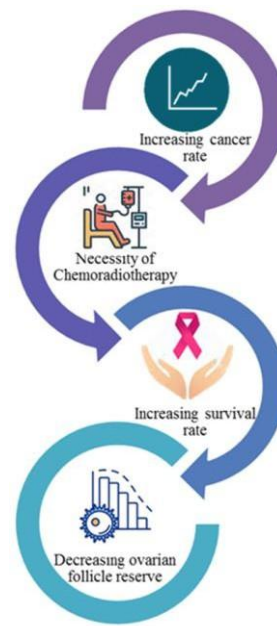
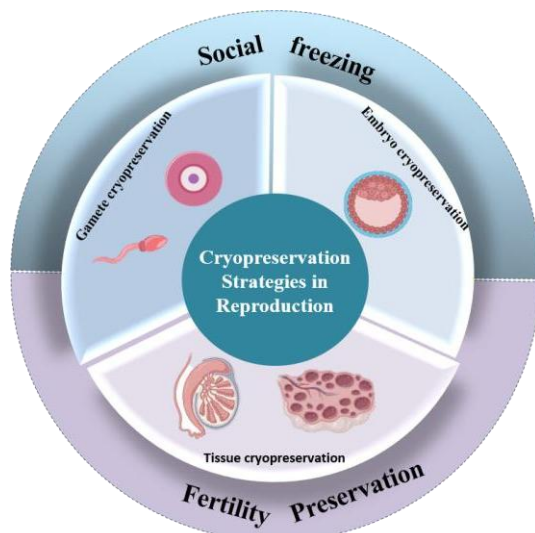


Figure 1. The necessity to preserve reproductive capacity.

Human fertility is impacted by other non-medical factors as well. Men and women marry later in life due to changed lifestyle expectations, financial constraints, and societal situations. Increased maternal age is one of the primary causes of foetal abnormalities and infertility (6). This is brought on by low-quality female gametes (egg) as women age, necessitating the preservation of fertility and healthy gametes created before to the age of 35. Currently, "social freezing" refers to the process of freezing oocytes, in vitro fertilised (IVF) embryos, or ovarian tissue in order to preserve younger women's fertility for motherhood. As a result, it seems that cryopreservation will be employed extensively in reproductive science in the years to come. This is because more people will apply for fertility preservation for non-medical reasons, which will inevitably boost freezing's economic attractiveness (7, 8).



New treatments have been developed and the demand for services in this field has increased as a result of raising public awareness of current fertility preservation techniques and expanding the knowledge of gynaecologists, oncologists, and other medical professionals related to infertility (Figure 2).

Figure 2. Different ways to select the best strategy in reproductive cells and tissues cryopreservation.

DIFFERENT METHODS TO PRESERVE HUMAN FERTILITY

Human fertility may be preserved in a number of ways, some of which are now considered "usual" approaches (9):

a) Hormonal support: The architectures of pharmaceutical and natural hormones are comparable. Hormonal support aims to preserve reproductive gonads during invasive therapies by suppressing or inhibiting gamete development.

b) Oophoropexy: in this case, the ovaries are moved beyond the radiation area within the pelvis. This approach is not always practical and can only be used to protect radiation-induced damage, not chemotherapy-induced harm.

c. Cryopreservation of gonadal tissue: this technique drastically lowers the gonads' physiological activity, and following thawing, testicular or ovarian tissues recover their normal functions. The loss of gonadal reserve brought on by cancer therapies, birth defects, and early ovarian failure may be prevented by freezing the testis and ovarian tissues.

As previously stated, the biological samples are frozen using both the slow freezing and vitrification methods. In the latter method, samples are quickly vitrified to stop the production of ice crystals, whereas in slow freezing, samples are gradually chilled in a programmed freezer. These days, these techniques are used in infertility clinics to freeze testicular, ovarian, sperm, eggs, and embryos (9).

Strategies for Cryobiological Preservation

The main goal of cryobiology as an applied science is to preserve biomaterials at low temperatures. Biological processes, including the apoptosis pathway, are at their lowest point at such low temperatures (13). Mammals' normal body temperature ranges from 32 to 37°C, and hypothermic storage is usually above 0°C. The idea behind hypothermic storage is that there is a 50% drop in oxygen consumption for every 10°C drop in temperature.

One way to avoid the acidosis linked to hypothermia in hibernating animals is to inhibit the activity of the sodium pump. Consequently,

Special solutions are used to counteract acidosis and raise intracellular calcium in order to preserve organs and tissues in vitro.

Three steps are usually involved in freezing methods: first, the samples are placed in a cryoprotectant solution (dehydration); second, they are submerged in liquid nitrogen (freezing or cooling); and third, they are brought back to room temperature (rehydration; thawing/warming). Despite having the same three stages, the two current freezing techniques slow freezing and vitrification, which can result in different cryopreservation outcomes for various samples (12).

CRYOPRESERVATION METHODS IN THE REPRODUCTION FIELD

Banking for ovaries, eggs, and embryos

While egg freezing is a popular fertility preservation technique for single women, embryo freezing is frequently the preferred method for women who have a partner (14).

Following the use of microinjection and in vitro fertilization in infertility treatments, the freezing of embryos became significant. The hormone pill used in IVF causes ovulation, which allows women to release up to thirty eggs. No ways to store extra oocytes or embryos, the IVF process used to be expensive and time-consuming. In the event that the current attempt fails, the remaining embryos can be stored for future attempts thanks to the development of freezing techniques, increasing the likelihood of pregnancy (15).

Costs and the physical and psychological stress brought on by repeatedly forced new stimulation cycles can be decreased by using the freezing technique. The number of embryos transferred to the uterus can be kept low using a range of cryopreservation techniques, which lowers the risk of multiple births and increases the number of eggs and embryos available for further treatments. Furthermore,

Only a certain number of embryos can be implanted in a woman under the proper conditions and at the right time. As a result, instead of forcing new stimulation cycles at different times, embryos can probably be transferred (15).

The safety of cryostorage techniques has been the subject of numerous studies. The outcomes have largely demonstrated the effectiveness and safety of current embryo cryopreservation techniques. In vitro fertilization produced the first baby in 1978 after the first successful cryopreservation of oocytes in mice was completed in 1977 (16). According to Trounson and Mohr (17), the first pregnancy and live birth from a frozen human embryo occurred in Australia in 1983 and attracted a lot of attention. After that, using frozen-thawed embryos became more common. A few studies on mouse oocytes led to the introduction of human ooc

ocyte cryopreservation into clinical practice in 1986 due to ethical concerns surrounding embryo cryopreservation (14). In 2006, both freezing methods were authorized as cryopreservation choices to preserve female cancer patients' fertility. Since then, freezing embryos and oocytes has produced over 5 million and 500,000 live births globally, respectively. Both mature and immature oocytes can be cryopreserved. The clinical outcomes of oocyte cryopreservation are dependent on a number of variables, some of which are related to the quality and health of oocytes and may be impacted by the cancer treatment procedure (14). Oocyte cryopreservation is primarily used for adult cancer patients to preserve fertility, particularly for young women who want to postpone getting pregnant. Oocyte cryopreservation can be used for young women who want to delay getting pregnant or for oocyte donation in place of embryos due to ethical concerns in some communities or the husband's absence (8). In embryology labs, mature oocytes can now be readily frozen using a variety of techniques, and numerous reports of live births have been documented (18). Nevertheless, the rate of live births using this technique is still extremely low, and the freezing of immature oocytes has not yet reached a satisfactory state. Oocyte survival after freezing has been somewhat improved by the application of novel methods like vitrification. The primary issue is the in vitro maturation of oocytes following freezing and thawing in relation to the laborious procedure of cryopreservation and oocyte retrieval. The likely inefficiency of the oocyte maturation medium is another issue. As a result, cryopreservation of immature oocytes has not yet proven to be sufficiently effective and requires further development and optimization research (14). For young single women and prepubertal girls with cancer, cryopreservation of immature oocytes is generally not advised and must be combined with the freezing of fragments of ovarian cortical tissues (19).

Oocyte cryopreservation is typically advised for patients who are single, but embryo cryopreservation would be the best and most efficient choice for women with a partner when there is sufficient time for stimulated ovulation. Cryopreserving ovarian tissues is suggested as a suitable method to aid in the preservation of fertility in situations involving ethical obstacles, the lack of ovulation induction, the urgency of cancer treatments, or the patient's immaturity.

(20). Ovarian tissue, which is a reservoir of follicles in different stages of growth and development, is cryopreserved so that cancer treatment can begin right away. Scientists have observed reports of successful live births due to the transplantation of frozen or thawed ovarian tissue, despite the fact that cryopreservation of ovarian tissue is currently being introduced to ART clinics as an experimental new method (21). Ovarian tissue freezing was suggested as a fertility preservation method for female cancer patients in 2018, following the first live birth from ovarian tissue cryopreservation and transplantation in 2004 by a Belgian group (Donnez and colleagues). Over 160 live births have been documented globally thus far (22). "We now find that the procedure of frozen ovary transplant is very robust," says Silber. Over 75% of patients become pregnant without IVF, and all patients have resumed hormonal function and cycling. As of right now, we have 23 healthy babies. If the ovary freeze is performed after the initial chemotherapy and definitely before the bone marrow transplant, it is safe even for patients with leukemia (23). Ovarian freezing is therefore regarded as a suitable technique for preserving fertility in cases of this kind of cancer involvement.

Banking for sperm

The quantity, motility, and morphology of sperm play a significant role in men's capacity to procreate. Male germ cells' susceptibility to

Because they can harm spermatogenesis and result in the total cessation of sperm production, infection, trauma, environmental factors like pollution from the workplace, and medications, particularly chemotherapy and radiation treatments, are important (24). Depending on the kind, quantity, and duration of exposure, men may become sterile as a result of chemotherapy, radiation, surgery, or a combination of these treatments used to treat cancer. Significant advancements in sophisticated IVF techniques have decreased the side effects of cancer treatment and stopped spermatogenesis. A workable and acceptable way to maintain fertility in cancerous men or those at risk of impaired spermatogenesis prior to any therapies is through sperm cryopreservation and storage in sperm banks. Advanced IVF and cryopreservation of sperm samples in liquid nitrogen at -196°C lessen the damaging effects of invasive chemotherapy and radiation on male germ cells. However, this method is not appropriate for young boys and is dependent on the presence of sperm. Testicular tissue preservation helps maintain fertility in these situations (25).

The establishment of a testicular bank

Numerous medications and treatments damage the testes, which results in the loss of testicular stem cells and

infertility. Therefore, one method for preserving fertility is to preserve testicular tissue before beginning any medication or radiation treatment. Children undergoing chemotherapy and radiation therapy may be able to conceive after puberty if their testicular tissue is preserved (24).

In patients with Klinefelter syndrome, cryptorchidism, many benign tumors (including myelodysplastic syndrome, anemia and thalassemia, autoimmune diseases, and lupus erythematosus), and particularly cancer in immature males, testicular tissue preservation is crucial. Patients with cancer are anticipated to live longer thanks to advancements in treatment methods. According to reports, only 33% of male survivors of childhood cancer have normal-quality semen, and their fertility is reduced by roughly 46% (26). Testicular germinal epithelium is highly susceptible to chemotherapy, particularly alkylating agents. These medications directly harm DNA and RNA and trigger apoptosis. Testicular somatic cells, such as Leydig and Sertoli cells, are less vulnerable to medications used in chemotherapy. Since radiation affects both somatic and germ cells, germinal epithelium is particularly vulnerable to its effects in addition to chemotherapy. In light of the harmful effects of radiation and chemotherapy on gonads, researchers are working to treat these patients with efficient fertility preservation techniques. However, this method is not appropriate for young boys and is dependent on the presence of sperm (27).

A testicular tissue biopsy is taken prior to any treatment for the aforementioned conditions, and the tissue is cryopreserved for later use. After testicular tissue cryopreservation, young boys can generally regain fertility using one of three methods:

1. isolating, freezing, and transplanting germ cells into the testis following gonadotoxic treatments;
2. the differentiation of isolated spermatogonial stem cells into spermatozoa in vitro;
3. After treatment, testicular tissue is frozen and re-implanted at the original location.

Testicular tissue cryopreservation has been the subject of numerous investigations in animals and, to a lesser extent, in humans. Spermatogonial cells of human testis were isolated and cultured using stem cells of testicular tissue, and the resultant colonies were first kept for six months in 2002 (28). Due to limitations, such as insufficient sample sizes, human testicular tissue has generally been the subject of few studies (25).

While cryopreservation and transplantation of ovarian tissue or cryopreservation of testicular tissue in prepubescent boys are examples of experimental reproductive techniques that should be centralized, only partial implementation is feasible in some areas and nations. Depending on their size and number of facilities, these centers' network structures differ significantly. Depending on the location, the centers' networks can range in size from small national networks like the Danish Network (www.rigshospitalet.dk) to extremely large national networks like the Oncofertility Consortium (www.oncofertility.northwestern.edu). In the areas of fertility preservation, continuing education and research, gathering high-quality and quantity data, and hosting national and international congresses, the creation of national or international networks can aid in bringing together experts, scientists, and students (29).

CRYOBIOLOGY HISTORY

The seasonal movement of flounders in cold water, the cold-hibernation of numerous organisms like tardigrades, and the survival of living things like psychrophile bacteria and polar plants at temperatures below zero all demonstrate that biological cells can withstand temperatures below freezing without altering their basic structure and functions. Because essential functions slow down at low temperatures, cells are shielded from enzyme and microbial deterioration and can be stored inactive for years before returning to normal upon rewarming. The study of biological materials or systems at temperatures lower than those typically encountered is known as cryobiology. Proteins, cells, tissues, organs, or entire organisms are examples of applied materials or systems of interest. As a result, the application of cryobiology science depends on various strategies, such as cell and tissue preservation and protection (30).

Microorganisms, plants, cells, tissues, and human and animal gametes and embryos have all been studied using cryobiology. Recent years have seen a great deal of research in the fields of cryobiology, including long-term storage, organ perseverance, cryosurgery, unhealthy tissue destruction techniques, food and

pharmaceutical freeze drying, and biological super-cooling (11).

Applied cryobiology has a long history that begins in antiquity. In 2500 BC, Egyptian medicine employed low temperatures (12). Hippocrates used cold to stop swelling and bleeding. Robert Boyle (1627–1691) investigated how animals were affected by temperature and wrote the first scientific paper on freezing at the beginning of modern science. About 70 years ago, cryobiology was first used in science to preserve biomaterials (31).

Numerous studies on freezing, cold hardiness, and frost resistance in plants were carried out in the late 19th and early 20th centuries. In order to determine what is necessary for survival or death following freezing, Hans Molisch investigated the impact of substance composition and concentration in plant cell cytoplasm in the 1890s (32). In the early 1900s, Maximov identified sugars as a cryoprotectant (33). In the ensuing years, researchers examined how insects and plant epidermal cells were affected by fast cooling and the biochemistry of cold hardiness.

Peter Mazur demonstrated in 1963 that cooling should be slow enough to allow enough water to escape the cell during the extra-cellular freeze dehydration process in order to prevent fatal intracellular freezing (34). A group of scientists under the direction of Christopher Polge cryopreserved bull semen for the first time in 1949 by adding glycerol to prevent freezing damage (35). Other solutes with high aqueous solubility were then studied as freezing-resistant substances. These chemical additives were referred to as "cryophylatic agents/solute moderators" (36). The Society for Cryobiology first referred to these additive substances as "cryoprotectants" in 1965. Since then, compounds that shield living tissues from harm after low temperatures have been referred to as "cryoprotectants" (12).

Three pregnancies resulted from artificial insemination with frozen human sperm in 1953 (37). Typically, human sperm, eggs, and embryos are kept for study and infertility treatment. The first frozen human embryo was used to conceive a child in 1983 after controlled-rate and slow-rate freezing techniques were established in the 1970s (17). The first ovarian tissue cryopreservation was carried out by Hovatta et al. in 1996 as a result of these studies' improved understanding of cryopreservation for the low-temperature storage of organs, tissues, and valuable cells (38). The first pup born from cryopreserved and transplanted mouse ovarian tissue was also accomplished in the same year (39). The first live birth following orthotopic transplantation of cryopreserved ovarian tissue in a cancer patient was accomplished in 2004 by Donnez and his associates (40).

Later, Silber et al. reported the first live birth in 2005 and created a new avenue for ovarian transplantation between monozygotic twins for POF (premature ovarian failure) (23).

In hospitals, veterinary clinics, and research labs, programmable devices have been widely used in recent years to freeze biological human and animal blood products, eggs, embryos, sperm, stem cells, and tissues. Large organs, like the heart, must be stored and transported for grafting right away after donation and only at low temperatures (not freezing). Tissue preservation's potential for transplantation was first recognized in 1970. by surgeon Thomas Starzl's work on liver transplantation (41).

CRYOPRESERVATION TECHNIQUES OPTIMIZATION

Cryopreservation can be harmful and fatal even though it maintains the structure of living cells and tissue (42). The formation of ice crystals that pierce the cell or the consequences of concentration changes of the composition in the liquid phase are the causes of the freezing damage (43). In addition to the intracellular area, the controlled slow cooling process causes ice crystals to form in the cell's extracellular region. The biggest drawback in this process is that the extracellular ice crystals may be dangerous (44). On the other hand, the toxicity and high cost of the cryoprotectant are the main drawbacks of vitrification. To avoid intracellular ice crystal formation in the vitrification process, high concentrations of cryoprotectants—which can result in osmotic damage—should be employed. However, it should be noted that both cryopreservation techniques employ cryoprotectants to decrease the formation of ice crystals by increasing the concentration of the total solute (45). Naturally, the sample type, volume, viscosity, and the rates of cooling and warming/thawing all affect how these cryoprotectants work (46).

Research indicates that vitrification-based embryo cryopreservation has advantages over slow-freezing. Because of this, slow-freezing reduces the metabolism of the embryo and affects its morphology, survival, and pregnancy rates. Furthermore, the vitrification method can result in fewer negative effects and higher rates of human embryo survival (47, 48).

On the other hand, research has shown that slow freezing is more effective and practical for cryopreserving human ovarian tissue. Apoptotic pathway-related genes are expressed in oocytes when human ovarian tissue is cryopreserved by vitrification. Additionally, compared to oocytes in ovarian tissue preserved by slow-freezing, the morphology of follicle oocytes is worse (49). Furthermore, reports regarding oocyte cryopreservation using vitrification or slow-freezing techniques are inconsistent. According to certain research, cryopreserved oocytes have a higher fertilization and blastocyst formation rate.

through slow-freezing (50, 29), though some disagree (51, 52, 53).

There have been reports of ultra-structural and sub-lethal sperm damage following cryopreservation. These injuries result in oxidative and osmotic stresses, which change the arrangement of lipids and proteins, damage the spermatozoa tail, and increase the fragmentation of sperm DNA (3, 19). Additionally, cryopreservation may result in structural damage to mitochondria that alters ATP production processes and reduces spermatozoa motility and viability, ultimately resulting in a failure in the quality of cryopreserved sperm (21).

As was previously mentioned, gametes age as a result of recent changes in general lifestyle that postpone marriage and childbearing. During the freezing process, older gametes may be more vulnerable to damage. Furthermore, research indicates that live birth and pregnancy rates are higher when fresh embryos are transferred as opposed to cryopreserved embryos, despite the advantages of cryopreservation for fertility and childbearing (54).

There is little knowledge about how cryopreservation techniques affect the physiology, cellular, and molecular levels (genomics, proteomics, and epigenetics) in embryos, germ cells, and gonadal tissues despite decades of use in fertility clinics (40). There are some findings despite the paucity of information in this area. Cryopreservation of the embryo, oocyte, and sperm can cause molecular damage related to metabolism, epigenetics, DNA integrity, cell death, and cell architecture. Following cryopreservation, reactive oxygen species are frequently elevated and cell viability is reduced. Both types of germ cells experience membrane impairment, but only sperm experience cytoskeleton impairment. The oocyte, sperm, and embryo exhibit mitochondrial damage, apoptosis, DNA fragmentation, and epigenetic changes. Furthermore, alterations in the lipidic profile and disruption of Ca^{2+} homeostasis are unique to the oocyte.

Consequently, a desired cryopreservation process results in a livebirth, and obtaining a healthy sample (with the capacity to grow in the lab or body) after thawing is regarded as a very important step. These days, a lot of scientists are working to find or create useful materials or tools for cryopreservation. Two factors need to be taken into account in order to optimize the cryopreservation process: a)

the components of the freezing medium; b) the equipment required to submerge and store samples in nitrogen. In the field of cryobiology research, techniques like the application of various cryoprotectant components, proteins, and freeze-drying are crucial, despite their secondary significance (42).

INNOVATIVE TECHNOLOGIES

Compared to other scientific domains, the availability of ART has increased significantly over the past three decades, leading to a number of advancements and innovations. The emergence of various ART in infertility treatment facilities, including IVF, ovarian stimulation, cryopreservation, and storage of male and female sex gametes, embryos, and reproductive tissues, is the pinnacle of these developments. These advancements are revolutionary in the treatment of infertile couples due to their applications in the diagnosis and treatment of infertility. All biological sciences, including research, clinical studies, cellular/molecular, and developmental aspects, have contributed to the current advancements in reproductive biotechnology. Recent accomplishments have produced capabilities that go beyond science itself. One result of advances in cryopreservation science in the field of reproduction is the creation of sperm, egg, embryo, and reproductive tissue banks.

A new branch of cryobiology research called assisted field cryobiology (AFC) uses a magnetic field to cryopreserve essential cells and tissues (43, 44). When combined with the use of nanoparticles, this strategy has produced intriguing results in the preservation of fertility at low temperatures (45, 55). Although

cryopreservation is widely used to maintain fertility in patients or those who wish to postpone childbirth for non-medical or medical reasons, other effective techniques have also emerged with outstanding results over the past ten years.

In the field of treating infertility, the use of ovarian stem cells or other stem cell sources to create male and female germ cells has become a top priority (56). In addition to mesenchymal stem cells derived from bodily sources like bone marrow, etc., the use of mesenchymal cells derived from embryonic stem cells is being investigated (57). Their direct injection into the One useful strategy for restoring reproductive activities in a variety of patients, especially those with premature ovarian failure, or POF, is ovarian tissue or blood flow (58). Male and female germ cells (eggs and sperm) can differentiate in vitro from adult stem cells derived from fat (46), peripheral (47), or menstrual blood (48), as well as endometrium or other tissues associated with the reproductive system or fetus structures, such as the amniotic membrane (50), umbilical cord (52), and peritoneum (57). These options can be useful substitutes for invasive methods of fertility preservation in patients, even though they are still in the experimental stage (53). For instance, ovarian tissue cryopreservation and/or ovarian follicle isolation techniques, which necessitate surgery and are regarded as invasive procedures, may be replaced by stem cells if they differentiate into germ cells and can be used in clinical practice (51).

In recent years, tissue engineering has gained popularity, and using "artificial gonads"—regenerated or stimulated in vitro ovaries and testes—as the ideal framework for producing male and female gametes (eggs and sperm) has become acceptable. These processes appear to have significant potential effects on future infertility treatment regimens (49). Researchers interested in ovarian tissue engineering now have more options thanks to recent successes in the reconstruction of mouse primordial follicles in the case of integrated mouse and human artificial ovary xenotransplantation (59) (Figure 3).

Figure 3. The new emerging techniques in the field of reproductive cryobiology.

A VIEW OF REPRODUCTIVE CRYOBIOLOGY'S FUTURE

We expect an intriguing and remarkable future for cryobiology in the field of reproduction science, even though a variety of fertility preservation techniques by tissue cryopreservation have been successfully implemented at renowned centers worldwide (Table 1). Significant prospects for future advancements are revealed by a thorough examination of the advancements made in the field of cryopreservation as reported in the most recent scientific literature, at conferences, and in other forums. We propose that the following will be the best measures of advancement:

- 1) Advancements in invasive chemotherapy and radiation techniques can stop side effects that result in infertility in both men and women. Fertility preservation and cryopreservation techniques can be used to avoid these negative effects (54).
- 2) Advances in short-term ovarian stimulation protocols to extract mature oocytes.
- 3) Ovarian transposition in cancer patients receiving radiation therapy, both inside and outside of the radiation field.
- 4) The use of hormonal medications to reduce ovarian reproductive activity in order to protect active organs exposed to dangerous and toxic chemotherapy medications. To put it another way, this technique lowers the ovary's production of gametes, or eggs, to shield it from harm during treatments (4).
- 5) The use of lyophilization to lower the initial costs of samples that are ready for freezing and likely transportation to other locations, as well as the additional expenses associated with long-term storage in liquid nitrogen.

Diseases like cancer, their treatments, and the rising age at which many people worldwide plan to start a family pose serious risks to reproductive fertility. The methods and strategies used to preserve fertility were covered in this paper, with particular attention to the storage of reproductive biological samples at low temperatures. After the current study, the Royan Institute (Tehran, Iran) further acknowledged the importance

of careful, well-organized planning and a well-documented strategic plan for the future of this science. It is hoped that this

Table 1. Examples worldwide of ovarian and testis cryopreservation centers.

Cryopreservation center	Country	Ovarian cryo	Testis cryo	Live births	*Cryo method	Reference
National Ovarian and Testicular Tissue Transport Cryopreservation Service (NOTTCS)	Australia & New Zealand			Five livebirths after grafting of ovarian tissue	SF	Fertility Society of Australia (FSA)
The Centre for Reproductive Medicine at UZ Brussel	Belgium			> 30 livebirths after grafting of ovarian tissue	SF	The Centrum voor Reproductieve Geneeskunde (CRG)
Laboratory of Reproductive Biology (LRB) Network	Denmark			15 livebirths after grafting of ovarian tissue	SF	www.rigshospitalet.dk
German-Austrian-Swiss FertiPROTEKT Consortium	Germany, Austria, and Switzerland			17 deliveries generated after 95 ovarian tissue transplantations	SF	(60)
Royan Human Ovarian Tissue Bank	Iran			Two unsuccessful ovarian tissue transplantations	V	(61)
Ankara University Medical Faculty, Center for Assisted Reproduction and Infertility	Turkey			One live birth following ovarian tissue transplant-ation	SF	(62)

*SF, slow freezing; V, vitrification

study can help towards improving the quality and quantity of public health and will be useful for all related research groups.

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