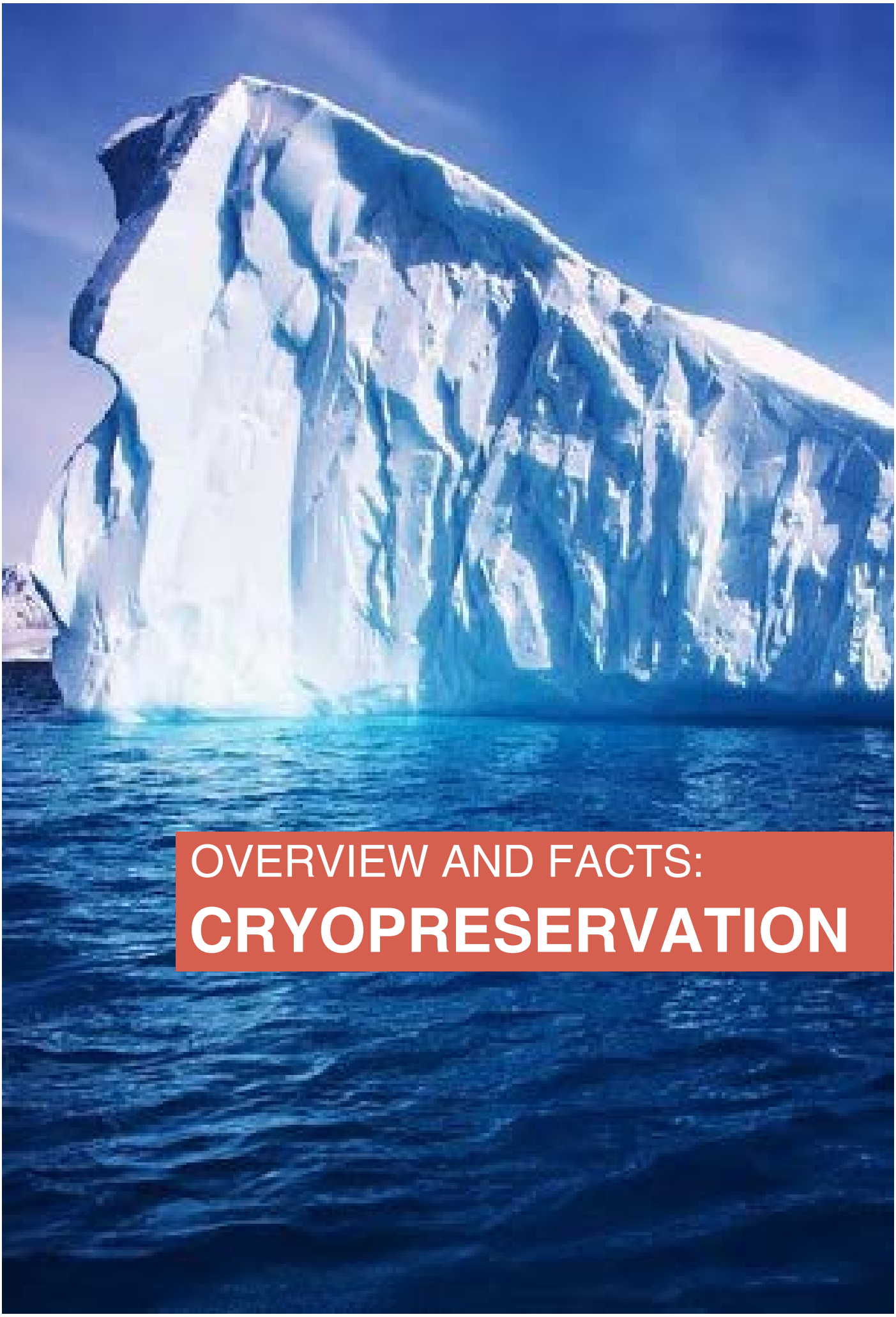

OVERVIEW AND FACTS:

# CRYOPRESERVATION

# Imprint

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By Victory A.R.T. Laboratory Phils, Inc.

This eBook was created by **http://www.ivfvictoryphilippines.com/** in hopes of helping bring into light infertility questions and bring solutions for many couples and / individuals having a hard time conceiving.



**Victory A.R.T. Laboratory Phils, Inc.** is part of the Hong Kong based Victory Group of A.R.T. Laboratories and is the pioneer IVF laboratory here in the Philippines. Established in and spearheaded by **Dr. Gregorio Pastorfide**, a re-knowned and internationally acclaimed OB-GYN, Victory continues to assist and provide infertility solutions using state-of-the-art equipment and an ever-growing and adapting medical team.

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# An Overview of Cryopreservation

Cryopreservation is the procedure that makes it possible to stabilize the cells at sub-freezing temperatures. Many advances in this technology have led to the emergence of methods that allow for long-term, low-temperature maintenance of sperm, ova, and embryos.

Cryopreservation is an integral part of assisted reproductive therapy, and is vital for the preservation of fertility for cancer patients, particularly before radiotherapy or chemotherapy.

Cryogenic storage appears to be the only proven solution that offers men a chance of having children in the future: cancer therapy and its adjuvants often lead to reproductive cell damage, resulting in subfertility or sterility due to gonad removal or permanent damage to germ cells. Even nonmalignant diseases such as diabetes and autoimmune disorders may lead to testicular damage. Cryopreservation is advisable in this regard.

For patients diagnosed with azoospermia (i.e. absence of sperm), cryostorage is also used to avoid having to repeat these procedures. Furthermore, cryopreservation is often performed in patients who want to freeze the semen sample preemptively to avoid inconveniences due to failed ejaculation.

Finally, sperm cryopreservation is recommended to preserve fertility in men who have a history of exposure to potentially toxic agents that may interfere with sperm production.

There are two main conventional freezing techniques used in sperm cryopreservation: slow freezing and rapid freezing. The slow freezing technique consists of progressive sperm cooling over a period of two to four hours in multiple steps using a programmable freezer.

The manual method is performed by simultaneously decreasing the temperature of the semen while adding a cryoprotectant in a stepwise manner followed by submerging the samples into liquid nitrogen.

The sample is cooled from room temperature to 5°C at a rate of 0.5–1°C per minute. Then the sample is frozen from 5°C to 80°C at a rate anywhere from 1–10°C per minute. Upon reaching -80˚C, the specimen is plunged into liquid nitrogen at 196°C.

Rapid freezing technique requires contact between the samples and nitrogen vapours for eight to ten minutes followed by immersion in liquid nitrogen.

The sample is mixed in with an equal volume of cold cryoprotectant; the mixture is loaded into straws and left to incubate at 4°C for 10 minutes. The straws are then placed at a distance of 15 to 20 cm above the liquid nitrogen level for 15 min. After this stage, the straws are submerged.

Cryopreservation of sperm requires the use of cryoprotectants: chemicals that are highly permeable and have low molecular weight. These are used to protect spermatozoa from damage from ice crystallization. Glycerol is the most widely used cryoprotectant for human sperm.

The thawing procedure is an equally important step: the cell must be allowed to recover its normal biochemical functions while avoiding abrupt thermal changes. The cryopreservation protocols use a thawing temperature of 37°C even if higher thawing temperatures allow for more rapid heating.

The risks associated with higher temperatures can lead to cell damage. Once the semen is thawed, it is separated from the cryopreservation medium by washing in culture medium and centrifuging.

# Cryopreservation Part 1: Oocyte Cryopreservation

Cryopreservation is a treatment for infertility that uses the latest in assistive reproductive technology to extract, freeze and store a woman's oocytes for the future. These eggs can be thawed, fertilized in vitro, and later transferred back to her uterus as embryos or blastocysts to complete their development.

Cryopreservation has also been applied to later stages in the life of a fertilized oocyte, with advances in the technology allowing for embryo and blastocyst freezing. The freezing of sperm from the male partner, as well as his testicular tissue, can also be preserved in this way.

***Egg Freezing: How it works***

The retrieval of eggs for oocyte cryopreservation begins with the administration of hormone injections to stimulate the ovaries to produce and ripen multiple eggs. When these eggs become mature, ovulation is induced, and the eggs are removed using an ultrasound-guided needle. This process is done while the woman is sedated, and the needle is guided through the vagina. Once the eggs are successfully harvested, they are immediately frozen.

Eggs are frozen by one of two methods: slow-freezing or flash freezing (vitrification). Cryoprotectants are added to replace the water within the oocyte and prevent ice crystals from forming and destroying the egg cell. The slow-freeze process is a cooling process that requires the eggs to be frozen at a controlled rate. On the other hand, vitrification is much faster, but requires higher concentrations of cryoprotectants to be added.

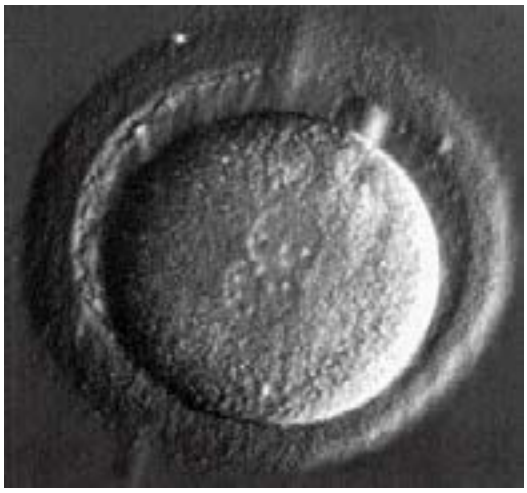
The result of the freezing process is a solid, glass-like cell devoid of ice crystals. Studies have demonstrated that vitrification leads to a higher survival rate and better development, while the slow-freeze method has been considered safer, leaving the eggs less prone to contamination or infection.

***Indications for Oocyte Cryopreservation***

Egg freezing is indicated in women who are currently undergoing assistive reproductive technology treatment, but do not wish to undergo embryo freezing. Some women consider freezing embryos to be against their religious beliefs or personal ethics. This ensures that no excess embryos are created and the mother is not faced with the decision to dispose of them, simply because they are not used.

Oocyte cryopreservation is also aimed at women who have been diagnosed with cancer who have not yet initiated radiotherapy or chemotherapy, since these treatment modalities are toxic for oocytes, and few eggs that survive are actually viable.

Women who simply want to postpone motherhood and preserve their future ability to have children, whether their reasons are medical, personal, or even professional in nature, can also benefit from egg freezing, and thus are allowed a chance a pregnancy when the time is right for them.



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# Cryopreservation Part 2: Embryo and Blastocyst Cryopreservation

***Embryo Freezing***

Similar to oocyte freezing, embryos and blastocysts are preserved at sub-zero temperatures. The embryonic stage of development corresponds to the pre-implantation, where under normal circumstances the fertilized egg is still within the fallopian tube en route to the uterus, where it would develop into a fetus.

After oocytes are harvested, they are fertilized using donor sperm and allowed to develop into embryos over three days. Embryo cryopreservation can be achieved via slow freezing or vitrification, much like what is achieved when oocytes are frozen.

Embryo cryopreservation is indicated for "leftover" embryos after the completion of an IVF cycle. During a single IVF cycle, many oocytes can be harvested and fertilized, but only a few are transferred back to the uterus. If the transfer results in a failed pregnancy, the other cryopreserved embryos can be transferred in a subsequent attempt. In the case of a successful transfer, the embryo can be transferred to the mother's uterus in the event she is ready for another pregnancy.

Embryos that have been created in the fertility treatment process may also be used for donation to another woman or couple trying to become pregnant.

The success rates for the transfer of cryopreserved embryos have been reported to be similar to fresh (i.e. non-frozen) embryos, with no increase in birth defects or other abnormalities.

Also, storage time has been shown to have negligible effects on the embryo survival rates, implantation, pregnancy, or live birth rates.

***Blastocyst Freezing***

A blastocyst is the subsequent developmental stage wherein the embryo is between five and seven days old. Compared to embryos, blastocysts are further along in their development, and are less vulnerable to some of the developmental pitfalls that may harm embryos. Additionally, since embryos are allowed two to four days to progress, the less viable embryos tend to fail, leaving behind their stronger cohorts, which are more likely to succeed. Embryos that fail to make it to the blastocyst stage usually have a chromosomal abnormality at fault.

Several studies have determined that blastocysts tend to have a better survival rate after cryopreservation than do embryos or earlier stages of development. Thus, blastocyst freezing has been considered to be superior to embryo cryopreservation.

The ability to thaw and transfer blastocysts on the same day is another advantage of blastocyst freezing that is not present in embryo cryopreservation. Embryos that have been thawed often must be allowed to develop into blastocysts prior to transfer into the uterus.

Similar to embryo cryopreservation, both slow freezing and rapid vitrification processes cam be performed, with vitrification appearing to be the successful, leading to higher survival and pregnancy rates.

Despite similar indications for blastocyst cryopreservation and embryo cryopreservation, many fertility clinics prefer cryopreservation of blastocysts due to their superior viability and higher success rates.

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# Cryopreservation: Sperm and Testicular Tissue Freezing

Aside from freezing ova, embryos and blastocysts, another option is to harvest reproductive cells and tissue from the male partner. Sperm and testicular tissues can be preserved, and this service is available for men who wish to bank their sperm for the future.

Cryopreservation of sperm and testicular tissue is especially useful for men who have been diagnosed with cancer, and face treatment modalities that are potentially harmful to their reproductive organs and cells. These treatments include chemotherapy and radiotherapy. Sperm freezing gives couples the option for fertility treatments such as intrauterine insemination and in-vitro fertilization in the future. This can be of great benefit to couples, especially if current circumstances are not favorable for conception.

Patients usually come in for consult after being referred by their health care provider. The consult is typically brief: your fertility specialist can handle any questions and clarifications you and your partner care to discuss.

***Sperm Freezing***

The male partner is asked to submit a semen sample, which is immediately warmed and kept at 37˚C. The sample undergoes liquefaction, and is then mixed with a freezing medium that allows it to survive freezing and storage. The freezing medium contains cryoprotectants, which remove water from the cells and prevent ice crystals from forming and causing cell death.

The samples of semen are frozen in vapors of liquid nitrogen and placed in cryovials for storage until they are needed. Each sample of ejaculate yields anywhere from one to six vials of semen.

The vials are labeled and catalogued for easy reference and retrieval.The survival rate for cryopreserved sperm is highly variable, thus it is recommended that one of the cryovials be thawed to assess the success rate and viability of cryopreservation.

Sperm are usually frozen for one year, however they can be frozen for longer periods if needed. Studies have shown that the likelihood of resulting in birth defects is the same for cryopreserved sperm and fresh ejaculate, and the chances of having a healthy newborn are indeed similar.

***Testicular Tissue Freezing***

The ability to preserve testicular tissue is important for maximizing the use of the tissue for sperm production. This treatment is of particular use to men who have cancer but still have viable testes, and wish to father their own biological children.

Testicular tissue freezing is a solution for treating azoospermia, a medical condition wherein a man has no measurable sperm in his semen. Although it is often caused by testicular abnormalities, azoospermia can be due to physical obstruction of the ejaculatory duct; in which case testicular function and sperm production are normal, and preserving this tissue is warranted.

Cryopreservation of testicular tissue can also be done for prepubertal boys undergoing cancer therapies that are toxic to their still-developing reproduc-

tive organs. Thus, it is a viable option in preventing male sterility.

After consulting with a fertility and assisted reproduction specialist, the patient undergoes screening for hormones, infection, and possibly chromosome testing. Then he is admitted for surgery, which can be performed under local or general anesthetic.

A small incision is made, and a small section of testicular tissue (approximately 2-3mm) is extracted for histologic examination and freezing.

The specimen is divided into several smaller sections, so that one can be tested for the presence of viable sperm. The other sections are frozen and stored in liquid nitrogen for future use, such as ICSI or in-vitro fertilization.

Here are some advantages of freezing testicular tissue:

The surgical procedure for testicular tissue biopsy is simple.

There is a low risk for infection and bleeding.
Surgery can be performed several months prior to any assistive reproductive therapy procedure, at the convenience of the male partner.

Microscopic evaluation of the tissue sample can give an accurate diagnosis for the reason behind azoospermia. If the biopsy specimen shows no sperm present, counseling for donor sperm or other options can be done immediately, avoiding unnecessary procedures to be done on the female partner.

# Benefits of Cryopreservation

Cryopreservation is a fertility therapy in which a woman's oocytes are extracted, frozen, and stored for the future. Later, the oocytes can be thawed, fertilized via in vitro fertilization, and later transferred back to the woman's uterus as embryos or blastocysts to complete their development into a fetus.

Cryopreservation has also been applied to later stages, such as in the development of a fertilized oocyte. Several advances in technology allowing for the freezing of embryos and blastocysts have made this possible.

The freezing of sperm from a male partner, or his testicular tissue, can also be done in cases where this is indicated. Cryopreservation in the Philippines is quite common, and is associated with in-vitro fertilization for patients having difficulty getting pregnant.

For women who are currently undergoing assistive reproductive treatment, but do not wish to freeze embryos, due to conflict with personal ethics or religious beliefs, oocyte Cryopreservationn is an optimal choice.

This ensures that there would be no excess embryos are created, thus the mother does not need to decide whether to dispose of unused, living embryos, or blastocysts further along in development.

Cryopreservation of oocytes also allows women who have been diagnosed with breast cancer and who have not yet started chemotherapy, radiotherapy, or other treatment modalities that would be toxic for their eggs.

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Also, women who simply choose to postpone motherhood until the right time, and preserve their future ability to have a baby can benefit from cryopreservation their oocytes.

For men, cryopreservation of sperm and testicular tissue is useful in cases where they have been diagnosed with cancer and face treatment that is potentially toxic to their reproductive tissue.
Sperm freezing gives couples the opportunity to try assistive reproductive therapies in the future. Freezing testicular tissue allows men to maximize the use of their testicular tissue for sperm production, and is a solution for treating azoospermia.

Cryopreservation of embryos and blastocysts also has some substantial benefits. In the event of a failed transfer of an embryo back to a woman's uterus, any available cryopreserved embryos can be transferred to her uterus. Thus, having more embryos available increases the chances of a successful pregnancy, and this is made possible through cryopreservation.

Embryos created in the process of assistive reproductive therapy can also be donated to another woman or couple who has tried and been unsuccessful at becoming pregnant.

Cryopreservation allows for several advantages that would otherwise be unavailable to patients seeking to get pregnant by natural, usual methods. It is integral in increasing IVF success rates in the Philippines, as it does in other parts of the world.

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*Success is not final, failure is not fatal; it is the courage to continue that counts.*
*- Winston Churchill*

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