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CRYOPRESERVATION OF SPERM AND OVUM

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ABSTRACT - Back ground of the study

Cryopreservation of sperm and ovum is an important Assisted Reproductive Technology (ART) used in the management of infertility arising from various causes, including cancer treatment with radiation, surgical procedures, occupational hazards, and other medical conditions that may affect fertility. Men between 18 and 41 years of age and women below 30–35 years can preserve their reproductive cells, namely sperm and ova, for future use. As today's youth are the future citizens of society, it is essential to educate students about the significance and benefits of cryopreservation of sperm and ovum. Such knowledge can help them make informed reproductive decisions and create awareness among others. Therefore, the present study was undertaken to evaluate the effectiveness of a planned Teaching Programme on knowledge regarding cryopreservation of sperm and ovum among nursing students of selected colleges in Bengaluru.

KEYWORDS: Assisted reproductive technology (ART), Effectiveness, Knowledge, planned teaching programme, cryopreservation of sperm and ovum.

INTRODUCTION

The sperm and ovum are the male and female reproductive cells, also known as gametes, which are essential for human reproduction. Sperm is derived from the Greek word “sperma” (meaning seed) and ovum is derived from Latin word Ova (meaning egg cell).

The sperm is produced in the testes of the male reproductive system, while the ovum is produced in the ovaries of the female reproductive system. Both sperm and ovum contain 23 chromosomes, which is half the number found in normal body cells. During fertilization, the sperm and ovum unite to form a zygote with 46 chromosomes, which later develops into an

embryo.

Fertility is the natural capacity to produce offspring; Infertility is failure to conceive within one or more years of regular unprotected intercourse. Primary Infertility means couples those who have never conceived. Secondary infertility means previous pregnancy but failure to conceive subsequently. There are many causes like defective spermatogenesis, errors in seminal fluid in males, anovulation, tubal damage, age above 35years in females etc.

Cryopreservation is one of the methods of fertility preservation that involves the cooling of a cells and storage at a temperature where all metabolic process is arrested.

Cryopreservation is based on the ability of small molecules to enter cells and prevent dehydration and formation of intracellular ice crystals, which can cause cells death and destruction of organelles during freezing process.

MEANING OF CRYOPRESERVATION

Cryopreservation is the process of preserving living cells, tissues, or organisms by freezing them at very low temperatures so they can be stored for a long time without damage. The procedure that makes it possible to stabilize the cells at cryogenic temperatures is called cryopreservation, also known as an applied aspect of cryobiology. Usually, the samples are stored at **–196°C in liquid nitrogen**, where all biological activities stop, keeping the cells alive for future use.

First banks for frozen human semen suggested in the year 1960. Cryopreservation of human spermatozoa introduced in the 1866's as an integral part of assisted reproduction technologies (ARTs). In fact, semen and ovum cryo storage is only proven method that may offer the couples a chance of having children in the future

PRINCIPLES OF CRYOPRESERVATION

Cryopreservation is the process of preserving cells, tissues, or biological materials at very low temperatures (usually

−196 °C in liquid nitrogen) so that their structure and function remain intact for future use.

The following are the main principles of cryopreservation

- **Low Temperature Storage**

Biological materials are stored at extremely low temperatures to stop all metabolic and biochemical activities. At these temperatures, cells remain in a suspended state without damage for a long period.

❖ **Prevention of Ice Crystal Formation**

Ice crystals formed during freezing can damage cell membranes and organelles. Therefore, techniques are used to control freezing so that large ice crystals do not form inside the cells.

❖ **Use of Cryoprotective Agents (CPAs)**

Chemicals such as Dimethyl Sulfoxide (DMSO) and Glycerol are added to protect cells during freezing.

These substances reduce ice crystal formation and help maintain cell integrity.

❖ **Controlled Rate of Freezing**

Cells are cooled gradually (usually about 1 °C per minute). Slow cooling allows water to leave the cells before freezing, preventing intracellular ice formation.

❖ **Rapid Thawing**

When the preserved sample is needed, it is thawed quickly to prevent the formation of ice crystals during warming and to maintain cell viability.

❖ **Maintenance of Sterile Conditions**

All procedures must be performed under sterile conditions to avoid contamination of the preserved biological material.

❖ **Proper Storage in Liquid Nitrogen Tanks**

Samples are stored in liquid nitrogen containers at −196 °C to maintain long-term stability and viability of the biological materials.

CRYOPRESERVATION OF SPERM

Cryopreservation of sperm is a medical technique used to preserve male reproductive cells at extremely low temperatures for a long period of time. This method is widely used in assisted reproductive technology (ART) to maintain fertility for future use.

The sperm cells are frozen and stored in liquid nitrogen at about −196°C, which stops all biological activity and keeps the sperm viable for many years.

Cryopreservation of sperm is the process of freezing and storing sperm cells at ultra-low temperatures in liquid nitrogen to preserve their viability and fertilizing capacity for future use.

Purpose of Cryopreservation of Sperm

❖ Fertility preservation: To preserve a man's fertility for future use, especially when he cannot conceive naturally later.

❖ Before cancer treatment: To store sperm before treatments like chemotherapy or

radiation, which may damage sperm production.

❖ Assisted reproduction: To provide sperm for use in In Vitro Fertilization (IVF) or Intrauterine Insemination (IUI).

❖ Low sperm count cases: To store good-quality sperm samples collected over time in men with low sperm count.

❖ Donor sperm banking: To store sperm from donors for use in fertility treatments for infertile couples.

❖ Delayed parenthood: To allow men to become fathers later in life when they are ready.

❖ Backup for Infertility Treatment – Provides stored sperm samples in case fresh sperm cannot be obtained during fertility procedures.

❖ Genetic or medical conditions: To preserve fertility before diseases or surgeries that may affect reproductive ability.

❖ For Surgical Sperm Retrieval – Sperm obtained through procedures like Testicular Sperm Extraction (TESE) can be frozen for future use.

❖ Long-term storage: Sperm can be stored for many years in liquid nitrogen at −196 °C and used when needed.

Criteria of sperm donors

- Age Criteria usually 18–41 years.
- Donor should be physically and mentally healthy.
- Donor should not have personal history or family history of inherited illness or abnormalities.
- Donors must free from the sexually transmitted disease like HIV, Hepatitis B, Hepatitis C Etc.

Recruitment

Recruitment of sperm donors is an important process in sperm banks and fertility clinics to obtain healthy semen samples for long-term storage and use in assisted reproductive techniques. Healthy men are invited to donate sperm through advertisements, medical institutions, or voluntary programs. Usually, donors are selected between the ages of 18 and 41 years and must be physically and mentally healthy with no history of genetic or hereditary diseases.

Before acceptance, donors undergo a strict screening process that includes medical history evaluation, physical examination, and laboratory testing for infectious diseases such as HIV/AIDS, Hepatitis B, Hepatitis C, and Syphilis. Semen analysis is also performed to assess sperm count, motility, and morphology. Only donors with normal semen quality are selected.

After screening, donors receive counselling about the procedure, ethical considerations, and confidentiality. They must give written informed consent before donating sperm. The collected semen samples are then processed and stored at very low temperatures using cryopreservation techniques for future use in infertility treatment.

Screening and Preparation of Sperm Donors Screening of Sperm Donors

- Collection of detailed medical and family history.
- Physical examination to assess overall health.
- Age requirement usually between 18 - 41years.
- Laboratory tests for infectious diseases such as HIV/AIDS, Hepatitis B, Hepatitis C, and Syphilis.
- Genetic screening for hereditary disorders like Thalassemia.
- Semen analysis to check sperm count, motility, morphology, and volume.

Preparation of Sperm Donors.

- Counselling and Informed Consent – Donor is informed about the procedure, risks, and legal aspects, and written consent is obtained.
- Abstinence Period – Donor is advised to maintain 2–5 days of sexual abstinence before semen collection to improve sperm quality
- Healthy Lifestyle – Donor should maintain balanced diet, proper sleep, and avoid alcohol, smoking, and drugs.
- Avoid Certain Medications – Some medications that affect sperm production may be restricted before donation.

- Semen Collection – Semen is usually collected by masturbation in a sterile container in a private room at the clinic or sperm bank.
- Sample Labelling and Identification – Proper labelling to avoid mix-ups or contamination.
- Semen Processing – The sample is tested and prepared for cryopreservation (freezing) using special cryoprotectants.
- Quarantine Storage – Frozen samples are stored for a period (usually 6 months) before final use.
- Repeat Health Testing – Donor is retested for infectious diseases before the sperm is released for clinical use.
- Documentation – All donor details, screening results, and sample records are maintained for traceability and safety.

Process / Steps Cryopreservation of Sperm

1. Patient Counseling and Consent

Before the procedure, the patient is informed about the purpose, benefits, risks, storage duration, and future use of the sperm sample. Written informed consent is obtained. Counseling is especially important for patients undergoing treatments like chemotherapy or surgery that may affect fertility.

2. Semen Collection

The semen sample is usually collected by masturbation into a sterile, wide-mouthed container in a private room in the laboratory or clinic.

- The patient is advised 2–5 days of sexual abstinence before collection.
- In special situations, sperm may be collected by testicular sperm aspiration (TESA) or epididymal sperm aspiration.

3. Semen Analysis

After collection, the sample is analysed in the laboratory to check:

- Volume of semen
- Sperm concentration (count)
- Motility (movement of sperm)
- Morphology (shape and structure)

This helps determine whether the sample is suitable for freezing and how many samples should be preserved.

4. Semen Preparation

The semen sample may be processed to remove debris, dead cells, and seminal plasma. Techniques like centrifugation or sperm washing are used to improve sperm quality before freezing.

5. Addition of Cryoprotectant

A cryoprotective agent (CPA) such as glycerol is added to the semen sample.

- Cryoprotectants protect sperm from damage caused by ice crystal formation during freezing.
- They also help maintain cell membrane stability and viability.

6. Equilibration

After adding the cryoprotectant, the sample is allowed to equilibrate for 10–15 minutes at room temperature. This step allows the cryoprotectant to penetrate the sperm cells and balance osmotic pressure.

7. Packaging and Labelling

The treated semen sample is transferred into cryovials or plastic straws. Each container is carefully labelled with:

- Patient name or identification number
- Date of freezing
- Sample number

Proper labelling prevents mix-ups and ensures safe storage.

8. Controlled Cooling (Pre-Freezing)

The semen sample is cooled gradually to avoid sudden temperature changes that may damage the sperm. This step may involve placing the sample in a programmable freezer or nitrogen vapor for controlled temperature reduction.

9. Freezing

After gradual cooling, the samples are frozen completely. The freezing process stops all metabolic activity in the sperm cells and preserves them for long periods.

10. Storage in Liquid Nitrogen

The frozen sperm samples are stored in liquid nitrogen tanks at −196°C. At this temperature, sperm can remain viable for many years without significant loss of function.

11. Thawing

When the sperm is needed, the sample is carefully thawed at room temperature or in a warm water bath. After thawing, the sperm can be used in assisted reproductive techniques such as:

- Intrauterine insemination (IUI)
- In vitro fertilization (IVF)
- Intracytoplasmic sperm injection (ICSI)

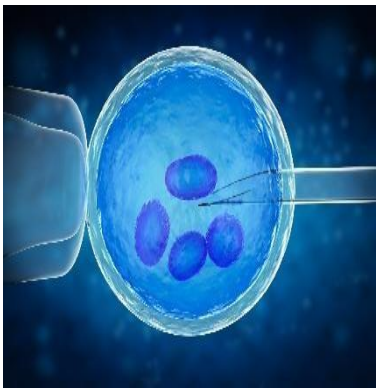
Duration/length of sperm storage

1. Short-term Semen Cryobanking is the depositing, freezing and storage of sperm at a sperm bank for less than one year.
2. Long-Term Semen Cryopreservation: According to the donor's specific wishes, these specimens can later be thawed and used. The statutory storage period is 10 years, but this can be extended with the consent for a total of 55 years. Every 10 years medical practitioner will need to validate the need for continued storage and provide a letter to extend the storage period

CRYOPRESERVATION OF OVUM

Cryopreservation of ovum is the process of collecting, freezing, and storing a woman's eggs (oocytes) at very low temperatures (usually –196°C in liquid nitrogen) to preserve fertility for future use.

It is an important technique in assisted reproductive technology (ART), allowing women to delay pregnancy or preserve fertility before medical treatments.



Purpose of cryopreservation ovum

- To preserve fertility for future pregnancy.
- To delay childbearing for personal, social, or career reasons.
- To protect fertility before chemotherapy or radiotherapy.
- To manage infertility in assisted reproductive techniques (ART).
- To store donor eggs for egg donation programs.
- To preserve fertility in women with premature ovarian failure risk.

- To safeguard reproductive potential in ovarian diseases (e.g., endometriosis).
- To reduce the need for repeated ovarian stimulation in IVF cycles.
- To allow women with genetic disorders to preserve healthy eggs.
- To provide reproductive options for women undergoing gender or medical treatments affecting fertility.

Types of ovum donors

a. Known Donors

Donor is known to the recipient (e.g., friend or relative)

b. Anonymous Donors

- Donor identity is kept confidential
- Commonly selected through fertility clinics or egg banks

c. Directed Donors

Donor is specifically chosen by the recipient (e.g., sister or cousin)

d. Shared Donors

Eggs from one donor are shared between two or more recipients

e. Altruistic Donors

Donate eggs voluntarily without financial benefit (except basic expenses)

f. Commercial Donors

Receive financial compensation for egg donation (regulated by law).

Criteria for Ovum Donors

- ❖ Age between 21–35 years
- ❖ Good physical and general health
- ❖ Regular menstrual cycle
- ❖ Normal ovarian function and good ovarian reserve
- ❖ No genetic or hereditary diseases
- ❖ Free from infections (HIV, Hepatitis B & C, syphilis)
- ❖ Normal hormonal levels
- ❖ Mentally stable and psychologically fit
- ❖ Healthy lifestyle (no smoking, alcohol, or drug abuse)
- ❖ Willingness with informed consent

Recruitment

Recruitment of ovum donors is carried out through fertility clinics, ART centres, and registered egg banks under strict guidelines. Donors may be identified through advertisements, donor registries, or voluntary applications. Interested women are provided with detailed Counseling about the procedure, risks, and legal aspects. Informed consent is obtained before proceeding. The process ensures confidentiality, ethical practices, and selection of suitable donors according to medical and legal criteria.

Screening and preparation ovum of donors Screening of ovum donors

- Detailed medical and family history
- General physical and gynaecological examination
- Screening for infectious diseases (HIV, Hepatitis B & C, syphilis)
- Hormonal tests (FSH, LH, AMH)
- Pelvic ultrasound to assess ovarian reserve.
- Genetic screening for inherited disorders.
- Psychological evaluation and Counseling.
- Blood group and compatibility testing.
- Lifestyle assessment (smoking, alcohol, drugs)

Preparation of ovum donors

- Counseling about the procedure, risks, and outcomes
- Obtaining informed consent
- Baseline medical examination and investigations
- Hormonal therapy (gonadotropin injections) to stimulate ovaries
- Regular monitoring with ultrasound scans
- Blood tests to check hormone levels
- Administration of trigger injection (hCG) for final egg maturation
- Advice on diet and healthy lifestyle
- Avoidance of smoking, alcohol, and drugs
- Preparation for ovum retrieval (fasting and pre-procedure instructions)

Process / steps of cryopreservation of ovum

Cryopreservation of ovum involves several well-planned steps carried out in assisted reproductive technology (ART) laboratories.

1. **Ovarian Stimulation**

- The donor/woman is given hormonal injections (gonadotropins) to stimulate the ovaries.
- This helps in the development of multiple follicles instead of a single egg.
- The process usually lasts 10–14 days.

2. **Monitoring of Follicular Growth**

- Regular ultrasound scans are done to monitor follicle size and growth.
- Blood tests are performed to measure hormone levels (estrogen).
- This ensures proper response to stimulation and prevents complications.

3. **Trigger Injection**

- When follicles reach the desired size, a trigger injection (hCG) is given.
- This helps in final maturation of the eggs.
- Ovum retrieval is planned about 34–36 hours after injection.

4. **Ovum Retrieval (Oocyte Pickup)**

- Performed under mild anaesthesia or sedation.
- A transvaginal ultrasound-guided needle is used to aspirate (collect) eggs from follicles.
- The procedure takes about 20–30 minutes.

5. **Identification and Selection of Oocytes**

- Retrieved fluid is examined in the laboratory.
- Mature and healthy oocytes are identified and selected for freezing.

6. **Cryoprotectant Addition**

- Special chemicals called cryoprotectants are added.
- These protect the eggs from damage during freezing by preventing ice crystal formation.

7. **Freezing Techniques**

a) Slow Freezing

- Gradual cooling of eggs over time
- Less commonly used now

b) Vitrification (Preferred Method)

- Ultra-rapid freezing method
- Prevents ice crystal formation
- Provides higher survival and success rates

8. **Storage in Liquid Nitrogen**

- Frozen eggs are stored in liquid nitrogen tanks at –196°C.
- Each sample is properly labelled and documented.

9. Thawing

- When the woman is ready for pregnancy, eggs are carefully thawed.
- Cryoprotectants are removed gradually to avoid damage.

10. Fertilization and Use

- Thawed eggs are fertilized using IVF or ICSI techniques.
- Resulting embryos are transferred into the uterus for pregnancy.

Duration/length of ovum storage

- Ova (eggs) can be stored for a long period, usually 10–20 years or more under proper conditions
- They are preserved in liquid nitrogen at –196°C, which stops all biological activity
- There is no significant deterioration in quality over time if storage conditions are maintained
- The duration of storage may depend on:
- Legal regulations of the country
- Clinic policies
- Patient's consent and requirement
- Eggs can be used whenever needed, as long as they remain properly stored

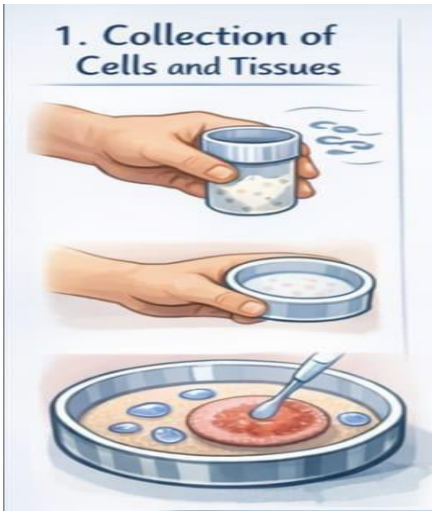
TECHNIQUES OF CRYOPRESERVATION OF SPERM AND OVUM

1. Collection of cells and tissues: -

- Collection is the first and most important step in cryopreservation, as the quality of the sample directly affects the success of preservation and future use.
- Sperm is usually collected by masturbation into a sterile container after a period of 2–5 days of abstinence, ensuring minimal contamination and optimal sperm quality. In cases where ejaculation is not possible, surgical methods such as testicular or epididymal aspiration are used to retrieve sperm. The sample is then allowed to liquefy and is evaluated for motility, count, and morphology before processing.
- Ovum collection, on the other hand, requires prior ovarian

stimulation using hormonal therapy to produce multiple mature follicles. Once the follicles reach the appropriate size, ovulation is triggered, and oocyte retrieval is performed after 34–36 hours using transvaginal

ultrasound-guided aspiration under aseptic conditions and mild anesthesia. The collected oocytes are immediately transferred to the laboratory, where they are examined for maturity and quality. Proper timing, sterile techniques, and careful handling are essential in both sperm and ovum collection to maintain cell viability for successful cryopreservation.



2. Addition of cryoprotectants

- The addition of cryoprotectants is a crucial step in the cryopreservation of sperm and ovum, as these substances protect cells from damage caused by ice crystal formation and osmotic stress during freezing and thawing.
- Cryoprotectants are broadly classified into permeating agents, such as glycerol, dimethyl sulfoxide, and ethylene glycol, which enter the cells and protect internal structures, and non-permeating agents, such as sucrose and trehalose, which remain outside the cells and help in controlling osmotic balance.
- During the procedure, cryoprotectants are added gradually to

the sperm or oocyte sample to prevent sudden osmotic shock that could damage the cell membrane. The cells are then allowed to equilibrate for a specific period to ensure proper penetration and distribution of the cryoprotectant. In sperm cryopreservation, glycerol is commonly used, whereas in oocyte cryopreservation, ethylene glycol and dimethyl sulfoxide are preferred due to their rapid permeability and effectiveness. The correct concentration and careful handling during this step are essential to minimize toxicity while maximizing cell survival during freezing and storage.

3. Freezing – (slow freezing method, Rapid freezing method and step wise freezing method.)

- Freezing is a critical step in the cryopreservation of sperm and ovum, as it determines the survival and viability of the cells during long-term storage.
- There are three main methods used: slow freezing, rapid freezing, and step-wise freezing.
- In the slow freezing method, the temperature is gradually reduced at a controlled rate, usually about 0.5°C to 1°C per minute, allowing water to move out of the cells and preventing intracellular ice crystal formation; however, it is time-consuming and may still cause some cell damage.
- In the rapid freezing method, also known as vitrification, the cells are exposed to high concentrations of cryoprotectants and then quickly plunged into liquid nitrogen, resulting in an ultra-fast cooling process that forms a glass-like solid without ice
crystal formation, making it especially effective for preserving ovum.
- The step-wise freezing method involves cooling the cells in a series of controlled stages, allowing gradual adaptation to decreasing temperatures and minimizing osmotic shock. Each of these methods aims to reduce ice crystal formation and preserve the structural and functional integrity of sperm and ova, ensuring better survival rates after thawing.

4. Storage

- Storage is a vital stage in the cryopreservation of sperm and ovum, where the preserved cells are maintained at ultra-low temperatures to retain their viability for future use.
- After the freezing process, the sperm or oocytes are stored in liquid nitrogen tanks at a temperature of approximately −196°C, which effectively halts all metabolic activities and prevents cellular degradation.
- The samples are placed in specially designed containers such as cryovials or straws, which are properly sealed and labeled with patient identification details, date, and type of specimen to avoid any errors.
- Strict monitoring of storage conditions, including maintaining a consistent temperature and adequate liquid nitrogen levels, is essential to ensure long-term preservation.
- Under optimal conditions, sperm and ova can be stored for several years without significant loss of viability. Proper documentation, handling, and safety measures are also important during storage to prevent contamination, damage, or accidental loss of samples.

5. Thawing

- Thawing is the final and equally important step in the cryopreservation of sperm and ovum, as it restores the frozen cells to a usable state while maintaining their viability and function.
- The process is usually carried out rapidly by placing the frozen samples in a water bath at around 37°C, which helps to prevent the formation of ice crystals during warming.
- After thawing, the cryoprotectants are carefully removed in a stepwise manner to avoid osmotic shock and cellular damage. The cells are gradually rehydrated and brought back to physiological conditions. Following this, the sperm are evaluated for motility, viability, and morphology, while

oocytes are assessed for membrane integrity and their ability to undergo fertilization.

- Proper and controlled thawing is essential because incorrect techniques can lead to cell rupture, reduced survival rates, and loss of function, thereby affecting the success of assisted reproductive procedures.

CRYOBANK OF SPERM AND OVUM

A cryobank is a facility used to store sperm and ovum at ultra-low temperatures for future use. The samples are preserved in liquid nitrogen at −196°C to maintain their viability. Before storage, sperm and ova are processed and mixed with cryoprotectants. They are then stored in sterile containers like cryovials or straws. Each sample is properly labeled for identification and safety.

Cryobanks have advanced storage tanks and temperature monitoring systems. Regular checks are done to maintain proper conditions. Strict aseptic techniques are followed to prevent

contamination. Proper records and confidentiality are maintained. Cryobanks are essential for fertility preservation and assisted reproductive techniques.

Today, the largest cryobank (sperm & egg bank) in the world is: Cryos International, located in Aarhus, Denmark. It is the world's largest sperm and egg bank with hundreds of donors and supplies to more than 100 countries.

India does not have a single officially declared "largest," but some major and well-known cryobanks include: Baby Quest Cryobank, Indogen Cryo systems, Cryobank India.

Bangalore does not have a single "largest" officially ranked cryobank, but several fertility centers and sperm banks provide cryopreservation services: Cloudnine Fertility (Multiple branches like in Whitefield, Jayanagar, Malleswaram, electronic city), Nova IVF fertility (Koramangala, Indiranagar, Whitefield), and other fertility centers also providing services.

COST FOR DONARS AND FREZZING

In USA/UK/ EUROPE, freezing cost is about Sperm freezing: $500 – $1,000 (₹40,000 – ₹80,000 approx.), Egg freezing: $6,000 – $15,000 (₹5 – ₹12 lakh approx.). Storage: $300 – $1,000 per year (₹25,000 – ₹80,000) and for Donor Cost is about Donor sperm: $300 – $1,000 per sample (₹25,000 – ₹80,000), Donor egg: $5,000 – $10,000 (₹4 – ₹8 lakh or more).

In India Freezing Costis about Sperm freezing: ₹5,000 – ₹20,000 (avg ~₹15,000), Egg (ovum) freezing: ₹1,00,000 –

₹2,50,000 per cycle, Storage charges: ₹10,000 – ₹25,000 per year and Donor Cost is about Donor sperm: ₹5,000 – ₹15,000 (approx.), Donor egg: ₹80,000 – ₹1,50,000.

In Bangalore for sperm freezing: ₹13,000 – ₹17,000, Sperm storage (yearly): ₹10,000 – ₹12,000, Egg freezing:

₹1,00,000+, Donor sperm: ₹5,000 – ₹15,000, Donor egg: ₹30,000 – ₹50,000.

IMPORTANCE OF CRYOPRESERVATION OF SPERM AND OVUM.

- Preserves fertility for future use.
- Maintains viability of reproductive cells for long periods.
- Useful in assisted reproductive techniques like IVF.
- Allows delayed parenthood.
- Helps individuals undergoing medical treatments affecting fertility.
- Enables use of donor sperm and ova.
- Provides backup for repeated fertility treatments.
- Prevents loss of reproductive potential due to age or disease.

- Offers flexibility in planning pregnancy.
- Supports individuals with infertility problems.

FACTORS TO BE CONSIDERED FOR CRYOPRESERVATION OF SPERM AND OVUM

- Quality of sperm and ovum (motility, count, maturity)
- Age of the individual
- Selection of suitable cryoprotectant
- Concentration of cryoprotectant used
- Rate and method of freezing
- Proper equilibration time
- Maintenance of ultra-low storage temperature (−196°C)
- Duration of storage
- Strict aseptic techniques
- Careful handling of samples
- Proper thawing technique
- Accurate labeling and documentation
- Prevention of contaminations
- Type of storage container (straws/cryovials)
- Monitoring of storage conditions

CONCLUSION

Cryopreservation of sperm and ova is a cornerstone of modern reproductive medicine, enabling long-term preservation of fertility through advanced freezing techniques such as slow freezing and vitrification. It plays a vital role in assisted reproductive technologies, including IVF, and offers individuals the opportunity to safeguard their reproductive potential in situations like medical treatments (e.g., chemotherapy), delayed parenthood, or donor programs.

Sperm cryopreservation is relatively simple, highly effective, and widely used, with good post-thaw survival and fertilization rates. Ovum cryopreservation, though more technically challenging due to the cell's size and sensitivity, has significantly improved with vitrification, leading to better survival and pregnancy outcomes.

Overall, cryopreservation enhances reproductive autonomy, supports fertility preservation, and contributes to advancements in genetic and reproductive research. Despite limitations

such as cost, need for specialized facilities, and potential cellular damage, it remains a safe, reliable, and increasingly successful technique in preserving human fertility.

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