IJNRD.ORG ISSN : 2456-4184
INTERNATIONAL JOURNAL OF NOVEL RESEARCH AND DEVELOPMENT (IJNRD) | IJNRD.ORG
An International Open Access, Peer-reviewed, Refereed Journal

"UMBILICA CORD TISSUE CRYOPRESERVATION"

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ABSTRACT:

Umbilical cord tissue cryopreservation involves collecting, processing, and long-term storing mesenchymal stem cells (MSCs) from the umbilical cord, offering a valuable resource for future regenerative medicine. MSCs have shown potential in differentiating into various cell types, such as bone, cartilage, and nerve cells, making them promising candidates for treating conditions involving tissue damage and degeneration. Unlike umbilical cord blood, which is primarily preserved for hematopoietic stem cells used in blood-related disorders, umbilical cord tissue preservation targets regenerative applications, including potential treatments for autoimmune disorders, neurodegenerative diseases, and tissue injuries.

KEYWORDS:

Umbilical cord tissue, Cryopreservation, Stem cell banking, Regenerative medicine, Cell therapy, Tissue engineering, Newborn stem cell preservation, Cord tissue collection, Cell differentiation, Stem cell storage, Stem cell viability

INTRODUCTION:

Umbilical cord tissue cryopreservation is a process that involves collecting, processing, and storing stem cells from the tissue of a newborn's umbilical cord at extremely low temperatures for potential future medical use(1-4). Unlike umbilical cord blood, which is primarily stored for hematopoietic stem cells used in blood-related disorders, the umbilical cord tissue contains mesenchymal stem cells (MSCs), which have the unique ability to develop into various types of cells, including bone, cartilage, muscle, and nerve cells. This property makes MSCs particularly valuable in regenerative medicine, where they are being researched for applications in treating conditions such as autoimmune diseases, neurological disorders, and injuries that result in tissue damage or degeneration(5-8).

The process of cryopreservation ensures that these cells remain viable for long periods, potentially providing families with access to personalized cellular therapies in the future. As interest in stem cell research grows, umbilical cord tissue cryopreservation has become an increasingly popular option for parents who want to preserve a resource that may hold significant therapeutic potential(9-11). However, while this technology shows promise, many of its applications are still in clinical trial stages, and important questions remain regarding long-term viability, ethical considerations, and accessibility(12-15).

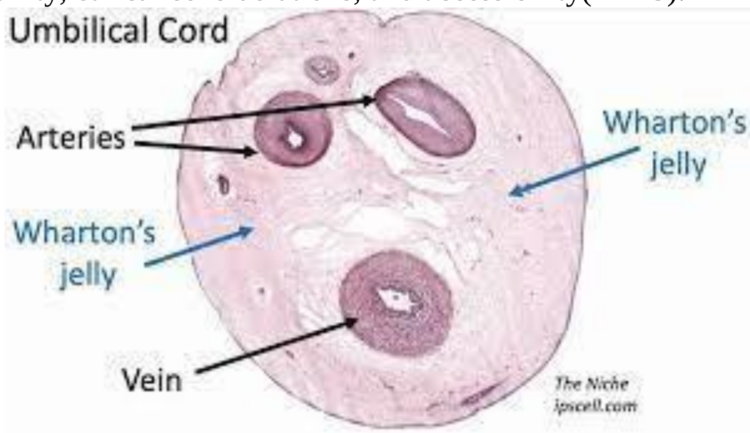

Fig – 1 Umbilical Cord(The figure is taken from Google)(Refe no.16-17)

PURPOSE OF UMBILICAL CORD TISSUE CRYOPRESERVATION

- The primary reason for preserving umbilical cord tissue is to have a potential resource for future therapeutic applications. These cells may be used in treating diseases and conditions that involve tissue damage or degeneration, including autoimmune diseases, neurological disorders, and injuries(18-19).
- Unlike hematopoietic stem cells (HSCs) from umbilical cord blood, which are primarily used for blood-related disorders, MSCs from cord tissue are valued for their regenerative properties(20-21).

Fig 2 – Newborn baby with Umbilical cord(Google)

CRYOPRESERVATION OF WHARTON'S JELLY:

Wharton's jelly (WJ) is a gelatinous substance found in the umbilical cord stroma, absent after birth. Named after Thomas Wharton, it protects umbilical blood vessels and ensures cord flexibility. WJ contains growth factors and extracellular matrix components like collagens (types I, III, IV, V), hyaluronic acid, and sulfated glycosaminoglycans, making it suitable for medical applications(22- 24).

Decellularized WJ has been used to create biomimetic scaffolds via freeze-drying, enhancing fibroblast growth and accelerating wound healing. WJ-based allografts, introduced in 2014, offer advantages over amniotic membrane grafts, including higher hyaluronic acid content and anti- inflammatory properties, promoting IL-

10 expression while reducing IL-12(25-26).

Cryopreservation of WJ does not preserve cells, and tissues are cooled to -80°C without cryoprotectants. WJ grafts have shown effectiveness in treating conditions such as spina bifida, severe ulcers, and wounds associated with diabetes and osteomyelitis(27-29).

Key Functions of the Umbilical Cord:

1. **Nutrient and Oxygen Supply:** Delivers essential nutrients and oxygen from the mother to the fetus through the umbilical vein, supporting fetal development(30-31).
2. **Waste Removal:** Transports waste products like carbon dioxide and metabolic byproducts from the fetus to the placenta via the umbilical arteries, allowing the mother to eliminate them(32-33).
3. **Immune Protection:** Transfers maternal antibodies to the fetus, especially in later pregnancy, providing initial immunity against infections after birth(30-32).
4. **Hormone and Growth Factor Transfer:** Acts as a channel for hormones and growth factors crucial for regulating fetal growth and preparing organs for life outside the womb(32-33).
5. **Shock Absorption:** Contains Wharton's jelly, a cushioning substance that protects the blood vessels from compression and maintains effective blood flow(33-34).

Anatomy of the Umbilical Cord

- **Umbilical Vein**: The single umbilical vein carries oxygen-rich blood from the placenta to the fetus.
- **Umbilical Arteries**: Two umbilical arteries return deoxygenated blood and waste from the fetus to the placenta.
- **Wharton's Jelly**: This gelatinous substance surrounds the vessels, providing support and protecting them from compression.
- **Cord Length and Flexibility**: The umbilical cord's average length is about 50-60 cm (20- 24 inches), which allows the fetus to move freely without causing damage or restricting blood flow.

Initiation of pregnancy with sperm that had been stored on dry ice for a short while was first done in 1953. The subsequent introduction of liquid nitrogen for the long-term cryostorage of sperm in the early 1960s substantially contributed to the efficacy of the approach. Contemporary cryotechnologies allow the long-term preservation of cells both in suspensions and within whole tissue fragments (e.g., whole adipose tissue, dental follicle tissue, bone marrow fragments, testicular and ovarian tissues), from which cells can be successfully isolated after thawing. Compared with the storage of isolated cells, the storage of unprocessed tissues has several advantages: minimization of time, labour, and material expenses; storage of cells in their natural environments; and future possibilities of cell isolation and expansion by as yet unknown future standards.

Full-scale experimental studies of UC tissue cryopreservation started about 10 years ago. Several types of UC cells, including epithelial and endothelial cells, are valuable for regenerative medicine and tissue engineering and can be cultured. Quite recently, an effective method for human umbilical vein endothelial cell (HUVEC) cryopreservation was reported importantly, the stage of cell culturing and expansion before transfer of the samples to the biobank is omitted in this procedure. Briefly, primary endothelium pellets, which are isolated from UC by enzymatic digestion, are frozen and placed in a liquid nitrogen freezer for long- term storage followed by fast thawing at + 37 °C. With this protocol, 14 viable HUVEC cultures have been successfully obtained from 17 primary endothelial pellets, which is an 82% success rate. The authors consider this approach helpful in improving the efficiency and logistics of biobanking, especially when processing large collections of endothelial samples(35-37).

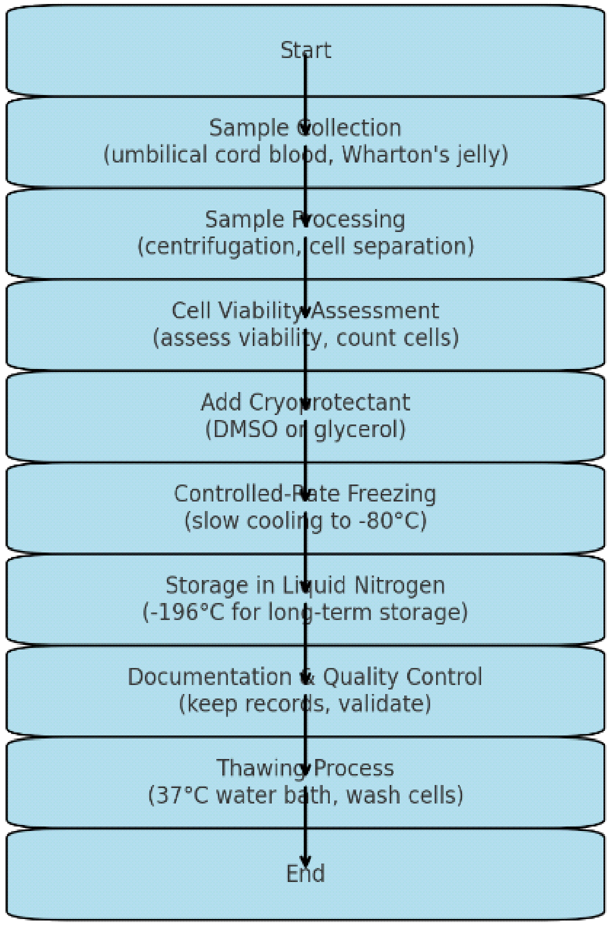

Fig 3 - Flow chart for Cryopreservation of Umbilical Cord component(Google)

THE PROCESS OF UMBILICAL CORD COLLECTION AND CRYOPRESERVATION:

The process of umbilical cord collection cryopreservation and banking involves several key steps, typically performed in a clinical setting immediately after childbirth. Here's an overview:

1. **Informed Consent**
   - Parents must provide informed consent before collection. They are usually given information about the benefits, risks, and uses of cord blood.
2. **Collection of Umbilical Cord Blood**
   - **Timing**: Collection occurs after the baby is delivered but before the placenta is expelled. This timing is critical to ensure the maximum volume of blood is collected.
   - **Method**: The healthcare provider clamps the umbilical cord and uses a sterile needle and collection bag or syringe to draw blood from the umbilical vein. The procedure is safe for both mother and baby.
3. **Transportation to the Laboratory**
   - The collected blood is labelled with the mother's and baby's information and transported to a cord blood bank or laboratory in a temperature-controlled container to preserve viability.
4. **Processing**
   - Upon arrival at the laboratory, the blood is processed to separate the stem cells from the plasma and red blood cells. This usually involves:
     - Centrifugation to separate components.
     - Testing for infectious diseases and ensuring the quality of the cord blood.

5. **Cryopreservation**
   - The separated stem cells are mixed with a cryoprotectant solution to prevent ice crystal formation, which can damage the cells during freezing.
   - The samples are then cooled slowly in a controlled-rate freezer to a temperature of approximately -196°C, where they are stored in liquid nitrogen.
6. **Banking**
   - The cryopreserved stem cells are stored in a cord blood bank. There are two types of banks:
     - **Public Banks**: Donated cord blood is stored for use by anyone in need. Parents typically do not pay for this service, but there is no guarantee their child will have access to the stored cells.
     - **Private Banks**: Families pay for the collection and storage of their child's cord blood for personal use, should the need arise in the future.
7. **Documentation and Follow-up**
   - Comprehensive records are kept of the collection, processing, and storage details. Parents receive documentation of the cord blood unit, including its unique identification number and testing results.
8. **Usage**
   - If needed, the stored stem cells can be thawed and used for treatments in various medical conditions, such as certain cancers, blood disorders, and genetic diseases.

Considerations

- **Ethical and Legal**: Parents should understand the ethical implications and legal rights associated with cord blood banking.
- **Medical Necessity**: Not every family needs to bank cord blood, so it's essential to weigh the potential benefits against the costs involved.

This process provides a valuable resource that can potentially save lives or treat serious health conditions in the future.

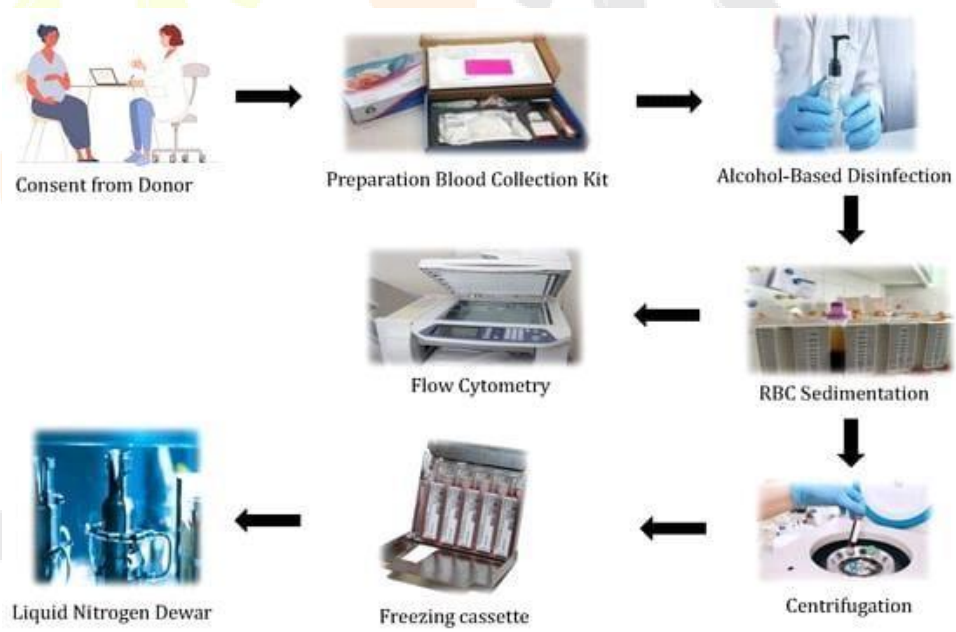

Fig 4 - The process of umbilical cord collection, cryopreservation and banking.

UMBILICAL CORD BLOOD COLLECTION:

Umbilical cord blood collection is a critical procedure for obtaining stem cells that can be used in various medical treatments and research. Here's a summary of the key aspects:

Collection Techniques

1. **In-Utero Collection:**

Performed before the placenta is delivered.

Preferred by most public banks in the U.S. and Europe.

Advantages:

Can be done by existing birth unit staff.

Requires no additional personnel or resources.

Yields higher volumes of cord blood and total nucleated cells. Involves a closed collection system, reducing infection risks.

2. **Ex-Utero Collection:**

Conducted after placental delivery.
Less commonly used in public banks due to logistical challenges.

In-Utero Procedure

1. **Preparation:**

The umbilical cord is double-clamped 3 to 5 cm from the umbilicus and cut between the clamps. After delivery, the cord is cleaned with a povidone-iodine applicator.

2. **Collection:**

The needle from the cord collection kit is inserted into the umbilical vein. Blood is collected by gravity into the collection bag.

3. **Time and Resources:**

Takes approximately 5–10 minutes.

Does not require additional staff or complex equipment.

This method is the standard for both private and public cord blood banks due to its efficiency and higher yield, making it essential for maximizing the utility of collected cord blood units (CBUs)..

PRIVATE CORD BLOOD BANKING:

Private cord blood banks store processed umbilical cord blood units (CBUs) for the exclusive use of the family who pays for the service. These CBUs can be used for hematopoietic stem cell transplantation or regenerative medicine therapies. The mother is usually named the legal custodian, and costs in Canada average $1,200 for processing and the first year of storage, with annual fees between $100 and $130. Some banks offer alternative payment plans, including an 18- year plan aligning with the child reaching adulthood.

Key Considerations:

1. **HLA Matching:** The likelihood of a sibling being a full match is 25%. Related CBUs may reduce the risk of graft-versus-host disease (GVHD) and improve transplant outcomes compared to unrelated CBUs.

2. **Cost Efficiency:** Using privately stored CBUs eliminates additional costs to the medical system, unlike public cord blood units, which can be expensive to obtain.

3. **Banking Criteria:** Private banks have less stringent collection criteria than public banks, accommodating smaller recipients. Emerging technologies aim to expand stem cell utility for larger recipients.

4. **Regenerative Medicine:** Cord blood is being studied for treating conditions like diabetes, brain injuries, cerebral palsy, and autism. Some trials require autologous CBUs, while others use allogeneic units.

Private banks appeal to families who foresee a specific medical need or want to preserve potential future options. However, therapeutic cell doses for regenerative medicine are still under development.

PUBLIC CORD BLOOD BANKING :

Public cord blood banks process and store donated umbilical cord blood units (CBUs) for potential use in hematopoietic stem cell transplantation. These banks are funded nationally or locally and provide CBUs for patients worldwide through registries, similar to bone marrow registries. Donated units are not reserved for the donor's family and must meet stringent quality criteria. Public banking is free for donors, but accessing CBUs internationally can be expensive, though costs are declining.

Key Considerations:

1. **Regulations & Standards:** Public banks in Canada must comply with Health Canada regulations and seek international accreditation (e.g., AABB, FACT) for quality and safety.

2. **Target Groups:** Public banking aims to support underrepresented ethnic minorities and patients with uncommon HLA haplotypes.

3. **Collection & Criteria:** CBUs must meet strict volume and cell count requirements to ensure transplant success. Many donated units are discarded or used for research.

4. **Costs & Funding:** Establishing and operating public banks is expensive, with costs typically borne by the public. International transplant centers may pay fees to access CBUs.

5. **Consent & Logistics:** Expectant mothers should provide informed consent for donation early, preferably before or early in labor. Hospitals and banking establishments manage consent, collection, and assessment processes.

Public cord blood banks prioritize creating a high-quality inventory for transplantation, but not all donations meet the strict criteria for storage and use.

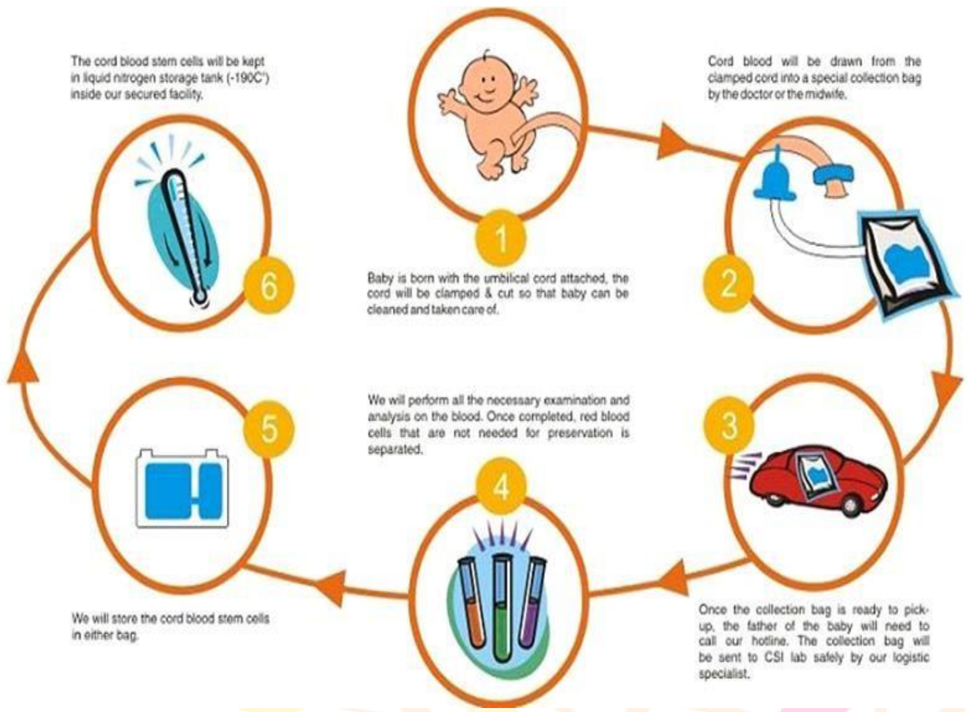

Fig 5 - A collection of umbilical cord

EDUCATION OF PARENTS AND HEALTH CARE PROFESSIONALS:

Despite increasing awareness of the therapeutic potential of umbilical cord blood stem cells, most pregnant women (70–80%) lack knowledge about cord blood banking and desire more information(38-40). While 80–90% of women prefer receiving information from healthcare providers, only a minority (15–30%) receive prenatal education or counseling. Instead, many rely on printed materials, the internet, or media. Surveys in Canada, Europe, and the U.S. indicate that once informed, most women are open to donating cord blood, with a preference for public over private banks(38,40,41).

While 80% of U.S. obstetricians feel confident discussing cord blood banking, fewer than 50% feel sufficiently knowledgeable to address patients' questions comprehensively. This lack of knowledge often leads to misinformation and confusion about the risks, benefits, and options for cord blood donation(38-39).

Women may choose public banks as an altruistic act of civic responsibility or private banks for potential family benefits. Since healthcare professionals are viewed as primary sources of guidance, they must receive adequate training and education to provide accurate and practical information about cord blood collection, storage, and banking options. Active involvement in prenatal education is essential to ensure informed decision-making by expectant mothers(41-43).

RESEARCH AND POTENTIAL FUTURE USE OF UMBILICAL CORD BLOOD:Umbilical cord blood is gaining attention for its potential in regenerative therapies and immune modulation. A systematic review of studies involving about 300 patients highlights its emerging applications, particularly in neurological repair, such as treating cerebral palsy(29-30). A large- scale study is currently exploring its efficacy in cerebral palsy treatment. Additional research suggests potential uses for conditions like type 1 diabetes and liver disease. Some studies are also examining the role of mesenchymal stromal cells expanded from cord blood in these therapies.

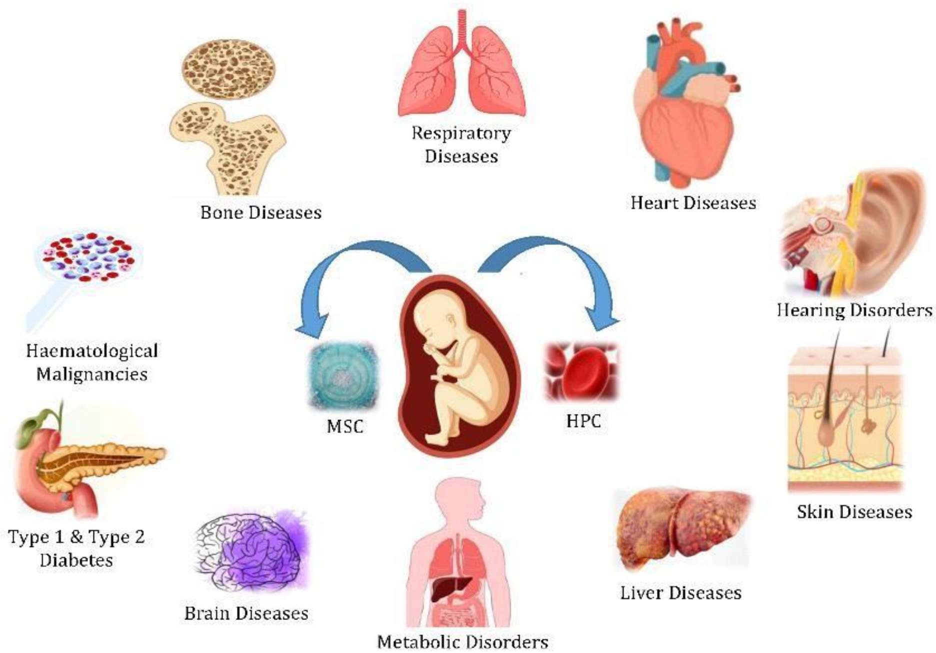

Fig 6 -Use of UCB stem cells in various disease conditions—UCB stem cells are currently used in a variety of clinical applications.

CONCLUSION:

The therapeutic use of umbilical cord blood (UCB) has significantly advanced over the past 30 years, despite ongoing challenges in collection, preservation, banking, and services. These challenges can be addressed through the diligent implementation of healthcare policies. A successful UCB process requires starting with counselling pregnant women about UCB donation and continuing until stem cells reach patients in need. Educating healthcare workers, particularly in prenatal and intranasal wards, about UCB procedures, ethical considerations, and collection techniques is crucial. Midwives and obstetricians should provide accurate information to parents during the antenatal period, ensuring informed decisions are made well in advance of delivery. This study highlights the importance of accessible, comprehensive information for healthcare professionals to enhance UCB practices.

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