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Cryobiology 3rd Symposium on and Biobanking

27 February, -2019
Royan Institute

Student award for the best thesis on cryobiology

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- Cryotechnology
- Cryomethodology
- Cryochemistry
- Cryobiophysic
- Cord blood stem cells
- Biobanking (human and animals)

Pre-Symposium Workshops
- Oocyte and embryo vitrification
- Ovarian Tissue Cryopreservation with Vitrification Technique
- Cell culture and cryopreservation
- Cryopreservation Techniques and Analysis of Sperm Parameters

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In The Name of God

3rd Cryobiology and Biobanking Symposium

27 February 2019

Royan Institute

Symposium Chairperson:
Dr. Bita Ebrahimi

Executive Secretaries:
Farideh Eivazkhani
Naeimeh Sadat Abtahi

Royan Reproductive Research Center
Embryology Department

3rd Cryobiology and Biobanking Symposium

27 February 2019

The **3rd Royan Symposium on Cryobiology** and biobank was held on **27 February 2019** in collaboration with the International Association of Society for Cryobiology in Royan Institute. The symposium is the result of more than two decades of research in reproductive biomedicine center of Royan Institute in the field of cryobiology. This scientific event intended to connect the science of cryobiology to other related scientific fields of study (i.e. biophysics and biochemistry). The symposium was an opportunity for co-thinking and knowledge enhancement while exchanging the latest achievements and the contemporary knowledge of cryobiology and biobanking. This year and during the symposium program, 12 national and international scientists presented their lectures and three pre-congress workshops were held with the following titles:

I. Oocyte and Embryo Vitrification

II. Cell Culture and Cryopreservation

III. Cryopreservation Techniques and Analysis of Sperm Parameters

189 participants joined the events and best student theses were selected and awarded in two graduate and postgraduate levels:

I. Evaluation of the structure and ultrastructure of fresh and frozen/thawed ovarian tissue in cancer patients, after culturing onto chick embryo chorioallantoic membrane (Mahboubeh Vatanparast; PhD).

II. Evaluation the changes in human sperm proteome and transcriptome with induction of mild oxidative stress in freeze-thaw process (Maryam Hazavei; PhD).

III. Effect of adding antioxidants Q10 and ellagic acid on freeze-thawing process of ram semen (Zahra Bolooki; MSc).sss

<u>The scientific and Administrative Secretariat:</u>

			
Dr. Rouhollah Fathi, PhD (Symposium Chair Man)	**Dr. Bita Ebrahimi, PhD Scientific Secretary**	**Farideh Eyvazkhani Executive Member**	**Naeimeh Sadat Abtahi Executive Member**

Scientific Program -3rd Cryobiology and Biobanking Symposium 27 February 2019, Royan Institute		
Dr. Bita Ebrahimi **(Scientific secretary)**	Welcome Message	**8:30 - 8:35**
Dr. Abdolhossein Shahverdi **(Royan institute chief)**		**8:35 - 8:40**
Dr. Gholamhossein Riazi	Microtubule Dynamicity in Cryopreservation	**8:45 -9:05**
Dr. Masoumeh Ramezanighara	What is Freeze Drying?	**9:10-9:30**
Dr. Hamid Mobasheri	Biophysics of Cryobiology, Facts in Freezing, Banking, Recovery and Treatment of Living Tissues at Molecular and Cellular Levels	**9:35-9:55**
Dr. Luciana Dini **(Video Lecture)**	Cryo-TEM and biochemical characterization of natural extracellular vesicles as nanocarriers for modulating immune responses	**10:00- 10:20**
Coffee Break		**10:20-10:50**
Dr. Iman Halvaei	The Efficacy of Antioxidants in Sperm Total Motility, DNA Damage, Viability and Reactive Oxygen Species Level During the Freeze-Thaw Process: A Systematic Review and Meta-analysis	**11:00-11:20**
Dr. Mojdeh Salehnia	Biology and Applicable Aspect of Female Sex Cells Cryopreservation	**11:25-11:50**
Dr. Seyedeh Zohreh Mirahmadi Zare	Nano-warming of Cryopreserved Biomaterials with Magnetic Nanoparticles Excited by Radio-Frequency	**11:55-12:15**

Lunch Break		**12:20-13:20**
Dr. Yaser Tahamtani	Production and Banking of Organoids for Disease Modeling and Drug Discovery	**13:30-13:50**
Dr. Fatemeh Hasani	Troubleshooting of a Cryopreservation System	**13:55-14:15**
Dr. Vida Sadat Kazemein Jasemi	Static-Magnetic Fields on Cryopreservation	**14:20-14:40**
Coffee Break		**14:45-15:10**
Dr. Masoume Nouri	Cryopreserving and Biobanking of Cord Blood Stem Cells	**15:15-15:30**
Dr. Seyed Abolhassan Shahzadeh Fazeli	Best Practice for Long Term Preserving of Biological Resource	**15:35-15:50**
Dr. Mohammad Hossein Ahmadi	Management of Microbial Contamination in Biobanks	**15:55-16:15**
Student Award Lecture		**16:20-16:30**
Closing Ceremony		**16:30-16:45**

Microtubule Protein Structure and Function in Proper Media

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Microtubule proteins structure and function have been described by many definitions. Such as a tubular protein functioning in signaling, movement, dipolar macromolecule memory chips in the brain and so forth. Microtubule proteins as an important and unique device for sperm movement has been determined. On the other hand, microtubules in sperm head are located, signaling for encounter preparation. So microtubule network seems to head the sperm though uterine. There for, changing normal polymerization and de polymerization. Temperature affect the novation function and intelligent network in sperm. Water molecules inside and outside the cell provide an electromagnetic network with microtubule proteins especially at the sperms tail. The electromagnetic network orientation is sensitive to the environment.

By applying special frequency of electro magnetite wave a special resistant network forms to the changes of temperature to freezing point. We examined polymerization of microtubules representing viability and functionality of sperms. The results demonstrated at special of electromagnetic frequency sperms would resist more freezing temperature than others.

What is Freeze Drying?

Ramezanighara Masoumeh, PhD
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Freeze drying is the removal of ice or other frozen solvents from a material through the process of sublimation. As a water removal process typically used to preserve perishable materials, to extend shelf life or make the material more convenient for transport. Freeze drying works by freezing the material, then reducing the pressure and adding heat to allow the frozen water in the material to sublimate. Lyophilization and freeze drying are terms that are used interchangeably depending on the industry and location where the drying is taking place. Controlled freeze drying keeps the product temperature low enough during the process to avoid changes in the dried product appearance and characteristics. It is an excellent dehydration method for preserving a wide variety of heat-sensitive and biological products such as proteins, microbes, pharmaceuticals, tissues & plasma. Sublimation in the freeze drying process can be described simply as:

1. FREEZE - The product is completely frozen, usually in a vial, flask or tray.

2. VACUUM - The product is then placed under a deep vacuum, well below the triple point of water.

3. DRY – Heat energy is then added to the product causing the ice to sublime.

In comparison with other drying processes, freeze-drying is considered as a reference for manufacturing high-quality dehydrated product. The direct transition of water from solid to vapor (sublimation), without a liquid phase, helps to preserve most of the initial raw material's properties such as appearance, shape, taste, color, and flavor. As an important functional property, the freeze-dried product has a high rehydration capacity. Consequently, except the application for biological active material (bacteria, vaccine), the use of freeze-drying is restricted in food industry to high added-value products like coffee, ingredients for ready-to-eat foods (fruits and vegetables, meat and fish), and aromatic herbs. Sublimation is a physical phenomenon by which solid ice is converted directly into vapor without it passing through the liquid state.

The steps required to lyophilize a product in a batch process can be summarized as follows:

- Pretreatment / Formulation

- Loading / Container (Bulk, Flask, Vials)
- Freezing (Thermal Treatment) at atmospheric pressure
- Primary Drying (Sublimation) under vacuum
- Secondary Drying (Desorption) under vacuum
- Backfill & Stoppering (for product in vials) under partial vacuum
- Removal of Dried Product from Freeze Dryer

Biophysics of Cryobiology, Facts in Freezing, Banking, Recovery and Treatment of Living Tissues at Molecular and Cellular Levels

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Presence of about 70% water with a unique behavior at low temperatures makes it vital in keeping the structure and function of organs, tissues, cells and even biological molecules in living systems in optimum state. Highly dynamic water molecules with femtosecond relaxation time, short life clusters and widespread dipolar effects create an activity pool where the dielectric and charged centers spatially and temporally change at high speed. The conformation and activities of the constituent biomolecules are greatly defined by the status of the pool and are highly susceptible to the temperature or better to say, the energy absorbed from intrinsic and external magnetic, electric, and electromagnetic fields. Application of our knowledge about structural, biological, physiological and bulk water as well as the Kosmotropic, Chaotropic agents, Cryoprotectants and Osmolytes at low temperatures has made it possible to consider wide spectrum of cryobiological manipulation and applications including; Cryopreservation, Cryogenics, Cryonics, Cryotherapy, Cryoablation, Cryosurgery, Cryotransport and so on. There is no doubt that awareness of the nano-environment situation at molecular level not only plays crucial role in the efficiency of the mentioned macroscopic approaches but opens new windows enabling us to tailor and fabricate novel advances towards preservation and manipulation of the living matters. Here, considering the biophysical facts and bioelectric of the living systems, the importance of temperature, the effect of its level and variation trend and rate on the structure, function, dynamics and integrity of the cells will be discussed and the involvement of water molecules in the formation of a supporting platform will be elaborated.

Cryo-TEM and Biochemical Characterization of Natural Extracellular Vesicles as Nanocarriers for Modulating Immune Responses

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Cryo-electron microscopy (cryo-EM) is a mainstream technology for studying the architecture of cells, viruses and protein assemblies, thus leading to major scientific breakthroughs and helping researchers to unravel the mysteries behind a wide range of diseases and biological mechanisms, becoming. Indeed, using cryo-EM, aggregate structures at molecular resolution within pristinely preserved cellular environments can be analyzed. In this study we used cryo-TEM to analyze the structure of the vesicles produced under physiological and pathological conditions by cells, i.e. Extracellular Vesicles (EVs), that according to size and origin, can be classified as exosomes (EXOs, 50-100 nm, produced via a lyso-endosomal pathway and released by an exocytosis mechanism) and microvesicles (MVs, 100-1000 nm, formed by budding of the plasma membrane).that They are natural shuttle vesicles, helped in this function by the specific molecules on their membranes that allow an easy interaction and internalization with a wide types of cells. EVs contain molecules of the originating cells (e.g., miRNAs, mRNAs and proteins) that pass into the recipient cells. For this properties EVs are very attractive in nanomedicine as natural nano-sized delivery vehicles, also able to enhance or suppress the immune system. Plasma membrane-derived MVs by expressing surface antigens may dinamically reflect the disease status and constitute a source of circulating biomarkers. On the other hand, EXOs, by belonging to the endogenous intracellular communication system, act as shuttle of nucleic acids and proteins from the cell of origin to recipient cells, are very attractive in understanding the biology of cancer microenvironment that contribute to the aggressive nature of GBM. In this work we characterize the structure of purified fractions of MVs and EXOs and we found that MVs induce an immunosuppressive microenvironment while EXOs favour an inflammatory response. The ability of EVs to modulate different biological responses opens new perspectives for a potential EVs therapeutic exploitation.

The Efficacy of Antioxidants in Sperm Total Motility, DNA Damage, Viability, and Reactive Oxygen Species Level During the Freeze-Thaw Process: A Systematic Review and Meta-Analysis

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This systematic review and meta-analysis aimed to detect the most effective antioxidant and identify the optimal dose to protect sperm total motility, viability, DNA integrity, and decrease Reactive Oxygen Species (ROS) level during the freeze-thaw process. A comprehensive search was done in PubMed, Embase, Web of Science, Scopus, and Cochrane electronic databases from inception until April 2018 without time limitations. Totally, thirty-one articles were analyzed. L-carnitine was identified as the most effective antioxidant in improvement of sperm motility (SMD=1.78, 95% CI:1.34-2.23) at the optimal dosage of 3.6 mM (SMD=3.93, 95% CI:3.05-4.81). Melatonin had the second position after L-carnitine to improve sperm motility and its optimal dose significantly improved this parameter compared to the control group. Tempol was the most effective antioxidant in protection of DNA integrity (SMD=-4.38, 95% CI:-5.17_-3.58) at the optimal dosage of 5 µM (SMD =-4.66, 95% CI:-5.77 - -3.55) and improved sperm viability (SMD=4.24, 95% CI: 3.66-4.81) at the optimal dosage of 50 µM (SMD=5.02, 95% CI: 3.69-6.34) during the freeze-thaw process. After tempol, vitamin E was the best antioxidant to protect spermatozoa's DNA against the detrimental effects of cryopreservation. Melatonin was recognized as the most effective antioxidant in ROS suppression (SMD=- 2.16, 95% CI:-2.36 - -1.96) at the optimal dosage of 0.01 mM (SMD= -10.84, %95 CI:-13.35 - -8.32). According to this study, L-carnitine was the most effective antioxidant in sperm motility, while tempol was most effective in reduction of DNA damage and improvement of sperm viability and melatonin was the most important antioxidant in ROS suppression.

Biology and Applicable Aspect of Female Sex Cells Cryopreservation

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The gametes, embryo and gonadal tissue cryopreservation have introduced for fertility potential preservation. Some of these techniques have become routine procedures into clinical practice and in human assisted reproduction such as sperm, oocyte and embryo cryopreservation. Embryo cryopreservation has resulted successfully method and increased the effectiveness of the IVF cycle. Oocyte cryopreservation offers more advantages in comparison with embryo cryopreservation, such as fertility preservation in unmarried girls whom need the oncological treatment. Embryo cryopreservation has also some ethical, moral and legal limitations. Various protocols have been developed for these purposes using different type and concentration of cryoprotectants, equilibration timing, cooling rates and cryopreservation devices. Two main protocols are: slow-freezing and vitrification. Ovarian tissue cryopreservation and auto-transplantation is another alternative method for restoring fertility in post-pubertal women and until now over 100 live births reported with a pregnancy rate of 23 to 37%. The in vitro maturation of follicles and in vitro culture of ovarian fragments are other possibility option that suggested for many women and girls.

Nano-warming of Cryopreserved Biomaterials with Magnetic Nanoparticles Excited by Radio-Frequency

Mirahmadi-Zare Seyedeh Zohreh, PhD
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Nowadays cryopreservation has become an indispensable part of assisted reproductive technique for cryopreservation of oocyte, sperm, embryos and tissue. The Synchronous success of the cooling and warming process ensures the success of the cryopreservation banking. Although there are various preservation strategies, no matter using tissue hydration, vitrification or other cooling methods, vitrification known as one of the best candidate for low volumetric cooling procedure. However, with increasing of cooling rate during the vitrification, the quality of cellular content will be maintained, but the standard warming protocols applied for thawing are essentially based on convective heat transfer and so slow. During standard warming protocol, sample is placed in a water bath or in air, but volume of the sample, heat conductivity of containers and low heating permeability of biological sample limit rapid and uniform warming in all over the sample. Convective warming of cryopreserved biomaterials in water bath remains the standard procedure for bio-specimens with less than 1 ml volume. Moreover, rate of warming by convection are below 50°C/min for vials with a radius of greater than 0.5 cm. Nanowarming through the fluctuation of magnetic nanoparticles in Ac magnetic field, recently attracts research attentions because of its potential applications in the oncology, cell therapy, transplantation, regenerative medicine and cryopreservation. Moreover, nanowarming is a technique that can warm cell and tissue systems from 0.01 to 80 ml. In this regard we discuss the current state of the magnetite applications, especially for warming of biomaterials, nanowarming performance, advantages and its limitations were measured in comparison with the various methods available for cell warming. In addition, parameters affecting the performance of magnetic nanowarming were classified according to available empirical reports.

Production and Banking of Organoids for Disease Modeling and Drug Discovery

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Organoids can be defined as micro-tissues which are developed in three-dimensional culture systems in vitro and contain tissue-specified cell types with the semi-realistic tissue geometry. In recent years, organoids have been produced by 3D primary culture of several human organ-derived cells including pancreatic cells. Human pluripotent stem cells (hPSCs) were also shown to be able to produce organoids due to their ability to self-renewal. These organoids were shown to be functional and proliferative even after long-term freezing. Such organoids are unique tools for studying human organ development, drug testing and cell replacement therapies. During the last five years, we have focused on studying the effect of co-culturing mesenchymal stromal cells (MSCs) with hPSC-derived pancreatic progenitors (hPSC-PPs) to produce functional pancreatic organoids. Our results showed that co-culturing hPSC-PPs with human fetal pancreatic derived mesenchymal cells (hFP-MCs) can improve the efficiency of endocrine development of PPs. We also introduce a method for production of pancreatic organoids (PO) which contain PP and MSCs. These PO showed functionality after transplanting to mice models. Although improvements are needed to define the optimal protocol for complex vascularized and functional generation of pancreas organ, it can be concluded that this co-culture principle can provide a powerful system to study human pancreas development biology and disease modeling.

Troubleshooting a Cryopreservation System

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With the rapid expansion in the use of assisted fertility treatments for the clinical management of infertility, a parallel increase happens in the cryopreservation procedure. There are two strategies that may fulfill the requirements for successful cryopreservation of mammalian oocytes and embryos: slow freezing and vitrification. In common with all other aspects of ART, troubleshooting in cryopreservation requires an understanding of basic principles of cryobiology. The practical tips for vitrification includes inverting the vials right before application to ensure proper mixing of the solutions and pre-equilibration of all the media for regulation of the temperature before dispensing. All vitrification devices should be labeled before use. Successful warming and vitrification hinges upon proper timing; thus, everything should be completely ready before the procedure is initiated. Since embryos tend to adhere to bubbles, no air bubble should exist on the surface of the initial warming solutions; this will hinder full submersion into the solution, and also affect the precise timing of the warming process. Vitrification technique could be exercised by abnormally fertilized embryos or unfertilized oocytes upon the consent of the patient prior to application in the treatment cycles. Paying a visit to a lab with a satisfactory vitrification program may also prove worthwhile. Slow freezing should be carried out with no bubble trapped in the freezing medium after the samples are put into the straws. Stable and accurate thermal control of the freezing device is crucial regarding the seeding temperature. Any alteration to the temperature in the controlled-rate freezer can lead to melting of the ice. As a safety measure, personal protective equipment (PPE), especially eye shields, must be used while thawing the straws. Cryostore capacity depends on the activity and the freezing capacity of the clinic, e.g., the number of cycles with annual freezing capacity. In addition, safety precautions should be observed for handling liquid nitrogen.

Vitrification Processes under Static Magnetic Field

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Preservation of fertility is an extensively researched topic, driven by the need to perpetuate life and preserve rare species of animals. Cryopreservation has emerged as an important method of fertility preservation, especially for storage of ovarian tissue, oocytes and embryos in female.

With regards to the cryopreservation of complex tissues containing multiple cell types, such as the ovary, the success of cryopreservation is dependent on the need to balance the freezing optima for a range of different cell types which are influenced by cell number and size and, in the case of oocytes, maturational status, as well as the requirement to preserve the structural integrity of the tissue. Cryopreservation can be carried out in a variety of ways. Researchers have used both slow freezing and vitrification procedures for ovarian tissue cryopreservation. Slow freezing methods aim to achieve the optimal balance between the rate of heat loss and extracellular ice formation. The disadvantage of the slow freezing is the formation of intracellular ice crystals. Consequently, vitrification methods have assumed greater importance in recent years than slow freezing processes. During vitrification, both intra and extracellular ice crystal formations are prevented due to high rates of cooling but the disadvantage of vitrification is the toxicity caused by higher concentrations of cryoprotectants used. To solve this problem, the use of a combination of permeable and non-permeable cryoprotectants like sucrose and ethylene glycol, has been suggested. Fast water exclusion from the cells could also reduce the toxicity effects of cryoprotectants. Magnetic fields have also been reported to enable accelerated release of water from cells/tissues, and thus prevent toxicity. It enhanced cryopreservation that has been considered in recent times as a promising type of ovarian or oocyte cryopreservation but the effectiveness of the process is still not clear.

Cryopreserving and Biobanking of Cord Blood Stem Cells

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Cell therapy is a promising therapeutic approach which has opened a clear horizon in the field of regenerative medicine. Stem cells derived from the cord blood and extra-embryonic tissues are important sources of stem cells with high applicable potential. Clinical application of these cells has led to the formation of an umbilical cord blood and tissue banking industry around the world. Since, in the most cases it takes several months or years the time from freeze to release, the use of stem cells in the right time depends on the proper and efficient storage of the cells through successful long term banking strategy. Therefore, keeping the cells in a frozen format while maintain obvious features of a functional cell is of great importance. Usually, the quality of stored stem cells is evaluated from different aspects like viability, clonogenic potential and expression of stem cell markers. Several factors interfere with the successful implementation of cryopreservation including the type of cryo-protectant agents, freezing protocol as well as equipment. We can overcome the challenges through understanding the physical and chemical mechanisms of cryopreservation to have a desired treatment based on stem cells.

Cryopreservation: Best Practice for Long Term Preserving of Biological Resource

Shahzadeh Fazeli Seyed Abolhassan, PhD
Iranian Biological Resource Center

Biobanking of biological resource, including viable cells, is a new and very relevant approach and is important to organize collection of high-quality samples with reliable clinical information for diagnostics, therapy and research. Biobanks play a crucial role in "-Omics" research providing well-annotated samples to study major diseases, their pathways and mechanisms. Cryopreservation, i.e. the storage of biological material at ultra-low temperature, usually that of liquid nitrogen (–196°C), is the only method currently available to ensure the safe and cost-effective long-term conservation of genetic resources. Cryopreservation of germplasm is of critical importance for operation of animal and human cell resource centers. Improved methods to efficiently and effectively cryopreserve germplasm are critical to provide assurance for reconstitution of a given strain when needed. cryopreservation has been successfully applied for germplasm conservation of a wide range of plant species e.g. rice, wheat, peanut, cassava, sugarcane, strawberry, coconut. Several plants can be regenerated from cells, meristems and embryos stored in cryopreservation.Cryoprotectants are the compounds that can prevent the damage caused to cells by freezing or thawing. The freezing point and super-cooling point of water are reduced by the presence of cryoprotectants. As a result, the ice crystal formation is retarded during the process of cryopreservation. Plant genetic resources (PGR) are important for agriculture and are mainly conserved in seed and field genebanks. Cryopreservation is an in vitro conservation method, which has become an important tool for the long-term storage of PGR.

Management of Microbial Contamination in Biobanks

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Contamination in biological materials (biospecimens) is a major drawback of biobanks throughout the world. It has cost of losing important biological products and/or valuable researches. The major sources of contamination include personnel, air, media, materials and reagents, glassware or apparatus (such as storage bottles and pipettes), and sample tissues or organs from which the cells were derived. The likely contaminants comprise different chemicals, bacteria (particularly *Mycoplasma* species), fungi, parasites, viruses, and even undesirable cell lines. The adverse effect of contamination on biological materials can be categorized into three classes concerning their significance including: (1) minor annoyances when up to several samples are occasionally lost due to contamination; (2) serious problems in which contamination frequency increases or entire biospecimens are lost; and (3) major catastrophes that call into doubt the validity of the past or current practice. The biobanks as well as other research centers have to monitor all processed biospecimens for possible sources of contamination to assure that no contaminants are introduced in the banking procedures. Hence, an efficient and reliable biobanking involves carrying out the routine microbiological screening of biospecimens and working in a controlled environment from beginning of collection to cryopreservation of the sample to reduce the probability of contamination in the final deposited product. Since some products banked are supposed to be applied for transplantation purposes to the patients and/or clinical treatments, the microbiological control programs look a vital step to be taken in order to avoid possible transmission of microorganisms to the recipients.

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