2nd Symposium

# Cryobiology and Biobanking

**Student award for the best photo and article on cryobiology**

**February 14 , 2018 - Royan Institute**

**Advances in basic and applied cryobiology in reproductive biomedicine and stemcells:**

- Cryotechnology
- Cryomethology
- Cryochemistry
- Cryobiophysic
- Cord blood stem cells
- Biobanking (Human and animals)

**In the name of Allah,**
**the most Beneficent,**
**the most Merciful**

## Message from Symposium Organizers

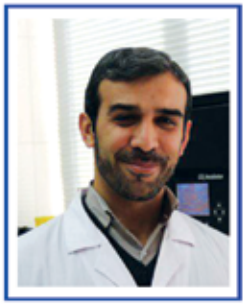
Dear Friends and Colleagues;

On the behalf of the organizing committee, I am delighted to welcome you to the 2nd symposium of Cryobiology and Biobanking in Royan Institute, Tehran, Iran. The scientific program includes 16 lectures by distinguished researchers covering multiple and vast variety of interesting topics concerned with cryobiology and biobanking. The main and core topics in this symposium are the advance ones both in basic and applied cryobiology, cryotechnology, cryobiophysic and Cord blood Stem Cells biobanking. Although, there is a broad spectrum of basic research presented, attention is also given to the clinical and industrial presentations. We strongly believe that the best status for satisfactory results is to integrate research findings into clinical and industrial experiment. Only under such a mutual interactions, we can witness the fruits of such progresses and achievements.

For the student award, the submitted research articles and photos are assessed according to their scientific novelty and impact. Then, we selected the best article and photo from the submitted articles and photos. I hope that you will enjoy this symposium, and find it a stimulating and informative meeting.

I would also like to remark the leading role Royan Institute is playing in the realm of new scientific research and developments it has brought into existence, bringing it glorious honor among other its counterparts. I hope, everyone enjoys this scientific gathering and event and find the lectures and articles of great future use.

Wishing you a best



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**Symposium Chairman**

**Dr.Mohsen Sharafi**
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## Executive Committee

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Elnaz Zand

## Scientific Program -2nd Cryobiology and Biobanking Symposium

**February 14, 2018, Royan Institute**

| | |
|---|---|
| **8:00-8:30** | **Opening** |
| Chairpersons: Dr. Zarrabi, Dr. Gourabi, Dr. Shahverdi | |
| **8:30- 8:50**<br>**Dr. Rouhollah Fathi** | Cryopreservation History in Royan Institute |
| **8:55– 9:15**<br>**Dr. Jason Acker** | Small Molecule Ice Recrystallization Inhibitors Control Ice and Protect Cells during Freezing and Thawing |
| **9:20-9:40**<br>**Dr. Saeed Abron** | Cord Blood Banking and Blood Disorders in Children |
| **9:45- 10:05**<br>**Dr.Mohammad Hossein Nasr Esfahani** | Both Cathepsin B Inhibitor and Vitamin K2 Can Improve Developmental Competency and Cryo-Tolerance of Ovine Embryos |
| **10:10-10:30**<br>**Royan Institute** | Royan Video Clip |
| **10:35-11:00** | **Coffee Break & Exhibition** |
| Chairpersons: Dr. Sadeghipanah, Dr. Alizadeh, Dr. Movaghar | |
| **11:00-11:20**<br>**Dr. Gholam Hossein Riazi** | Structure and Function of Macromoleculars in Freezing Temperatures |
| **11:25-11:45**<br>**Dr. Bita Ebrahimi** | Fertility Preservation in Female |
| **11:50-12:10**<br>**Dr. Mahdi Zhandi** | Nutritional Approaches for Improvement of Sperm Cryopreservation |
| **12:15-12:35**<br>**Dr. Shahin Ahmadian** | Cryo-electron Microscopy in Structural Biology |
| **12:40-13:30** | **Lunch Break & Exhibition** |

| | |
|---|---|
| Chairpersons: Dr.Ebrahimi, Dr. Noori, Dr. Nikoogoftar | |
| **13:30-13:50**<br>**Dr. Armin Towhidi** | Sperm Nanocryopreservation in Domestic Animal |
| **13:55 - 14:15**<br>**Dr. Jason Acker** | Donor Factors Have a Critical Role in the Cryobiological Response of Cells, Tissues and Organs |
| **14:20-14:40**<br>**Dr. Ensiyeh Hajizadeh** | GMP-compliant Biobanking |
| **14:45-15:05**<br>**Dr. Marziyeh Tavalaee** | The Role and Importance of Antioxidants in Sperm Freezing |
| **15:10-15:30**<br>**Dr. Mahdi Habibi** | Quality Management System of Biobanking |
| **15:35-16:00** | **Coffee Break & Exhibition** |
| Chairpersons: Dr. Imani, Dr. Shahhosseini , Dr. Eftekhari-Yazdi | |
| **16:00-16:20**<br>**Dr. Mahdi Hajian** | Effect of Cold Storage of Ovary on Handmade Somatic Cell Nuclear Transfer Outcomes |
| **16:25-16:45**<br>**Dr. Elyas Ariyakia** | Plant Cryopreservation |
| **16:50- 17:10**<br>**Royan Institute** | Sperm, oocyte and embryo Cryopreservation (Video Clip) |
| **17:10-18:00**<br>**Royan Institute** | Student Award |

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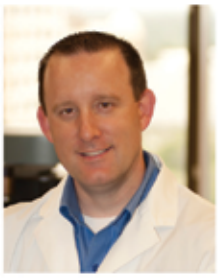

# Small Molecule Ice Recrystallization Inhibitors Control ice and Protect Cells during Freezing and Thawing

Tracey R. Turner[1], Jennie G. Briard[2], Jessica S. Poisson[2], Chantelle J. Capicciotti[2], Robert N. Ben[2] and Jason P. Acker[1,3]

[1] Canadian Blood Services, Edmonton, AB, Canada
[2] Department of Chemistry, University of Ottawa, Ottawa, ON, Canada
[3] Laboratory Medicine and Pathology, University of Alberta, Edmonton, AB, Canada

Ice recrystallization is a significant contributing factor to cell injury, resulting in reduced cell viability following cryopreservation. Consequently, the ability to inhibit ice recrystallization is a very desirable property for an effective cryoprotectant and has become an important strategy for improving cell viability. Recently, we have demonstrated the use of small carbohydrate-based molecules to function as ice recrystallization inhibitors (IRIs) and our laboratory has developed these molecules as cryoprotectants. In vitro studies using human red blood cells (RBCs) and hematopoietic stem cells (HSCs) demonstrate that these ice recrystallization inhibitors are effective cryoprotectants. Using cryomicroscopy tools we have been able to show how IRIs protect against extracellular solute toxicity injury and from intracellular ice damage. This presentation will provide an update on the use of IRIs to protect cells and tissues from recrystallization injury which occurs during transient warming events or during thawing. The potential for using IRIs in reproductive cell (oocytes and embryos) cryopreservation will also be discussed.

# Cord Blood Banking and Blood Disorders in Children

Saeed Abron

Department of Hematology, Faculty of Medical Sciences , Tarbiat Modares University , Tehran , Iran.

After a baby is born and the umbilical cord is cut, some blood remains in the blood vessels of the placenta and the portion of the umbilical cord that remains attached to it. After birth, the baby no longer needs this extra blood. This blood is called placental blood or umbilical cord blood: "cord blood" for short.

Cord blood contains all the normal elements of blood - red blood cells, white blood cells, platelets and plasma. But it is also rich in hematopoietic (blood-forming) stem cells, similar to those found in bone marrow. This is why cord blood can be used for transplantation as an alternative to bone marrow.

Cord blood is being used increasingly on an experimental basis as a source of stem cells, as an alternative to bone marrow. Most cord blood transplants have been performed in patients with blood and immune system diseases. Cord Blood transplants have also been performed for patients with genetic or metabolic diseases. More than 80 different diseases have been treated to date with unrelated cord blood transplants.

Cord blood offers a number of advantages to donors and transplant recipients. It is easy to collect, often more likely to provide a suitable match and is stored frozen, ready to use.

1.Cord blood collection is easy and poses no medical risk to the mother or newborn baby.
2 .Cord blood is collected in advance, tested and stored frozen, ready to use.
3. Cord blood transplants do not require a perfect match.
4. Cord blood transplants are associated with lower incidence of GvHD.
5. Cord Blood Transplants are associated with lower risk of viral infections.

# Both Cathepsin B Inhibitor and Vitamin K2 Can Improve Developmental Competency and Cryo-Tolerance of Ovine Embryos

Mohammad Hossein Nasr-Esfahani

Department of Cellular Biotechnology, Cell Science Research Center, Royan Institute for Biotechnology, ACECR, Isfahan, Iran.

Vitamin K2 (VK2) acts as an electron carrier in mitochondria and thereby effects reactive oxygen species (ROS) and ATP production. Furthermore, cathepsin B is a lysosomal cysteine protease involved in apoptosis. This study evaluated roles of VK2 and cathepsin B inhibitor, E-64, on *in vitro* developmental competency and cryo-survival of pre-implantation ovine IVF derived embryos. Initially, the optimal and beneficial concentration of VK2 (0.1 µM) and E-64 (1.0 µM) on compaction and blastocyst formation rates was defined. Subsequently, it was shown that 0.1 µM VK2, at blastocyst stage, reduces H2O2 production, increase the expression of mitochondrial related gene and improved embryos quality. In addition, we showed that 1 µM E-64, reduced DNA fragmentation and BAX as apoptotic markers while increasing total cell number per blastocyst and improving anti-apoptotic marker, the BCL2. We, further, assessed presence VK2 supplementation before and/or after vitrification of *in vitro* derived blastocysts. Our results revealed that presence of VK2 before and after vitrification improves rates of blastocysts re-expansion and hatching compared to control group. Furthermore, we showed that addition of 1.0 µM of E-64 during day 3 to 8 of development improved re-expansion and hatching rates of blastocysts post vitrification. E-64 also reduced rate of DNA fragmentation and BAX expression and increased total cell number per blastocyst and BCL2 expression post vitrification. However, addition of E-64 post vitrification reduced the hatching rate. Therefore, we showed that VK2 supplementation post genomic activation (Day 3-7) improved

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## Structure and function of macromolecules in freezing temperatures

Gholam Hossein Riazi

Institute of Biochemistry and Biophysics, University of Tehran, Tehran, Iran.

Water is the most recognized matter from the early time of human existence on the earth. It is believed that man considers water as a living fluid and well-being. However, we know it is a good feature but for special circumstance at 37°C and one atmospheric pressure particularly when we observe it in macro level. Since the molecule of water in macro-environment affects macromolecules such as proteins, lipids, and polysaccharides, it should be different in micro-environment such as **quantum tunneling behavior** of water monolayer around cells membrane. Water molecules would bind to hydrophilic agents on the membrane. But it is imaginable that water is concentrated around proteins more than lipid bilayers. The hexagonal structure of water molecules builds a cylinder like molecules where is able to change the membrane structure which makes membrane deform, as at crystal level at freezing temperature. While thawing the cell culture by opening up the distance between water molecules, the fragile membrane breaks and bond or free proteins are affected at the first time. To save the protein initially in the free form of bonded, the hexagonal structure of water must be interrupted. Interruption of water semi-crystal network helps to make defection on water structure sonication applied for the disruption of water around the microtubule protein where is the major constituted of the sperm tail. The question raised here is:"Which is the driving force to lead the sperm for a straight directed movement?" The results demonstrated that using sonication force disrupting semi-crystal water network followed by disabling the water to make the hexagonal structure and so the protein was protected of denaturing.

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# Female Fertility Preservation

Bita Ebrahimi

Department of Embryology, Reproductive Biomedicine Research Center, Royan Institute for Reproductive Biomedicine, ACECR, Tehran, Iran.

Nowadays, with the advances in medical science, cancers have become curable diseases with on time diagnosis and the patient can return to normal life after chemo/radiotherapy. Chemo/radiotherapy can cause gradual degeneration of primordial follicles in the cortical part of the ovarian tissue OR tissues and disturb ovulation procedure which leads to infertility. In addition to cancers, non-malignant diseases including blood diseases, some benign diseases such as recurrent ovarian endometriosis, life style changes and increased mean age of marriage decreased the fertility period of women and highlighted the importance of planning for fertility preservation.

Embryo cryopreservation for married women and oocyte cryopreservation for single adults are the first available fertility preservation options while there is enough time for ovulation induction without delay in cancer treatment. In the cases such as impossibility of malignancy treatment delay, pre-pubertal girls, hormone sensitive malignancy and etc, mentioned techniques are not suitable and ovarian tissue OR tissues cryopreservation is the only available option. In this method, the ovarian tissue is isolated by laparoscopy, laparotomy or uni/bilateral oophorectomy, then cryopreserved and stored. After cancer treatment procedure, this tissue could be transplanted back to the patient.

Among different methods of cryopreserved ovarian tissue usage, transplantation of frozen-thawed ovarian tissue is the only method which has led to live birth till now. It should be emphasized that there are some problems when using this technique including: necessity of a surgery for isolation of ovarian tissue, the risk of reintroducing malignant cells specially in ovarian cancers and low success rate. 60 live births have been reported following transplantation of frozen-thawed ovarian tissue till 2015 in all around the world. There is high probability of returning ovarian tissue activity if the cryopreserved ovarian tissue contains primordial follicles and the ovarian tissue functionality sustains over 11 years by repeating ovarian tissue transplantation. It is noteworthy that fertility preservation techniques in female malignancies are only valuable when uterus was preserved during surgery.

## Using Nutritional Supplements to Improve Sperm Cryopreservation

Mahdi Zhandi

Department of Animal Science, Collage of Agriculture and Natural Resources, University of Tehran, Karaj, Iran

Cryopreservation is a method to maintain spermatozoa for a long time and using them for artificial insemination and in vitro fertilization both in humans and animals. It has been well defined that freezing–induced osmotic and oxidative stresses have negative effects on DNA, mitochondria, plasma membrane structure, transport and survival in the female reproductive tract and function of spermatozoa. Frozen spermatozoa can be used for patients after procedures that would impair or curtail fertility and genetic improvement of agriculturally important species like dairy cattle. Ameliorating cryo-injury effect of freezing process has been considered by numerous studies in the last decades. However, most of them have focused on in vitro strategies to modify the effectiveness of some extenders. Using different types of antioxidants and cryoprotectantso, or even different cooling rates, are among those strategies to improve post-thawed sperm quality. Recently, using nutritional supplements has been established as a new strategy to improve sperm freezing ability. In most of studies, dietary effects of poly- unsaturated fatty acids with different sources have been evaluated on sperm freezing ability. The results of our studies on bull and ram showed that fish oil-supplemented diet resulted in increased motility and viability of post-thawed spermatozoa. However, in some studies no significant effects of poly- unsaturated fatty acids were observed and this discrepancy is probably due to a difference in source of oil or animal species. More recently, we showed that oral administration of Chrysin and D-aspartic acid could improve motility, mitochondrial activity, fertility, plasma membrane integrity and functionality of post-thawed rooster spermatozoa. In conclusion, it seems that more studies are required to evaluate the efficiency of dietary effective supplements on sperm cryopreservation.

## Cryo-Electron microscopy in Structural Biology

Shahin Ahmadian

Institute of Biochemistry and Biophysics, University of Tehran, Tehran, Iran

Understanding the function of cellular machines requires a thorough analysis of the structural elements that underline their function. Electron microscopy (EM) played a key role in providing information about cellular ultrastructure, as well as macromolecular organization. Nowadays, Single-particle cryo-electron microscopy (cryo-EM) is experiencing an unprecedented expansion, producing structures at faster and higher resolution. However, it is important to recognize that the recent surge in single-particle EM structures is not just the result of the newly developed direct electron detection device (DDD) cameras, but is the result of many methodological and technical advances. Important progress made over the years includes the discovery that quick-freezing preserves biological specimens in a near-native environment, with the subsequent development of specimen vitrification eliminating limitations on the achievable resolution which were imposed by the older specimen preparation techniques of metal shadowing and negative staining. Vitrified specimens, in turn, required the development of cryo-specimen holders and low-dose imaging procedures. Electron microscope also continued to improve, first featuring higher acceleration voltages, and then field-emission electron sources and improved electron-optical systems. Image detection also advanced from film to charge-coupled device (CCD) cameras, which facilitated high-throughput data collection but had inferior data recording characteristics. The recently introduced DDD cameras now deliver images of unprecedented quality, and changed the way cryo-EM data collected (movies instead of single exposures) and revolutionized what can be accomplished with cryo-EM. Using these techniques, one can acquire three-dimensional (3D) information about the macromolecular structural of cells, depict unique cellular states and reconstruct molecular networks.This presents an exciting opportunity to explore the molecular structure of both individual cells and multi-cellular organisms of nanometer to sub-nanometer resolution.

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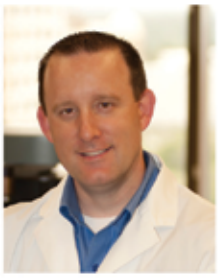
# Nano-cryopreservation of Sperm in Domestic Ruminants

Armin Towhidi, Touba Nadri, Mojtaba Mousavi

Department of Animal Science, College of Agriculture and Natural Resources, University of Tehran, Karaj, Iran

Research has shown that soy lecithin is a suitable alternative to animal-based components in extender, but typically is less efficient than egg yolk or milk for animal sperm cryopreservation. During past years, we used nanotechnology to increase the efficiency of soy lecithin in domestic ruminant's cryopreservation extenders. Research conducted in ruminant animal showed that in viability in extender containing 3% lecithin Nano micelles were higher compared to extenders containing 3% micro micelles and a commercial extender AndroMed in Bovine sperm (respectively, 73.94%, 71.12% and 61.90%). Moreover, progressive motility and viability of goat sperm in extender containing 2% Nano micelles (47.83%, 68.87%) were higher compared to extenders containing of 2% micro micelles (33%, 55.75%) and egg yolk (47.16%, 64.06%) respectively. This is probably due to smaller particle size (nanometer) and increased surface to volume ratio of the particles and thus efficient interaction and protection of sperm during freezing and thawing process. In a new study, different ratios of Nano-micelles to Nano-liposomes (3:0, 2.5:0.5 and 2:1 for Nano micelles of lecithin: Nano-liposomes of lecithin) were used to freeze Bovine sperm. Extender containing of 3% lecithin (2:1 Nano micelles: Nano liposomes) had the highest percentage of progressive motility (49%) and viability (80.09%) compared to other treatments. Recent data indicated that increasing Nano-liposomes ratio in the extender, improved post-thaw quality of sperm. Overall, it seems that the use of liposomal structures alongside the micelles and the particle size below 100 nm could increase the efficiency of lecithin as a cryoprotectant in ruminant sperm diluents.



## Donor Factors Have a Critical Role in the Cryobiological Response of Cells, Tissues and Organs

Jason P. Acker, MBA PhD
Laboratory Medicine and Pathology, University of Alberta, Edmonton, AB, Canada

Donor factors have long been associated with clinical outcomes in transfusion and transplantation, ranging from fundamental transfusion practices such as ABO/Rh blood group matching and pathogen screening to more current concerns such as the increased risk of TRALI associated with plasma products from female donors. Recently it has come to light that donor factors such as age and sex may also influence the *in vitro* quality of stored cell therapies. As the permeability of cells to water and solutes is critical in defining the optimal cryoprotectant additional and removal conditions and optimal cooling and warming rates for cell therapies, any donor-specific changes to the membrane may impact post-thaw recovery and quality of cells or tissues cryopreserved using a "standard" protocol. This presentation will review our current work examining the utility of different technologies to examine pre-cryopreservation factors that influence the physical characteristics of cells. The impact of donor factors on the ability for cells to respond to the physical and chemical stresses encountered during cryopreservation will be introduced. While there is a practical / commercial need to optimize donor selection and the pre-cryopreservation manufacturing efficiency of cellular products, consideration must be given to the impact and dependency that donor factors and the manufacturing conditions have on the quality and potency of the final product. Understanding the role that donor factors play in the cryobiological response of cells, tissues and organs may be critical in optimizing storage strategies and transfusion / transplant outcomes.

# GMP-compliant biobanks

Ensiyeh Hajizadeh-Saffar

Department of Molecular Systems Biology, Cell Science Research Center, Royan Institute for Stem Cell Biology and Technology ,ACECR, Tehran, Iran.

A collection of biological material and the associated data and information stored in an organised system, for a population or a large subset of a population is specified as biobank. Until recently, most biorepositories were primarily used for archival sample storage. However, in the last ten years, the industry landscape has evolved. With the introduction of advanced biotherapeutics and cell therapy products, biorepositories have transformed from archival storage facilities into true biobanks where the overall workflow now includes numerous complex transactions. The development, administration, and storage of a cellular therapy or biologic involves many moving parts. This is not due to regulatory demands being intensified, rather as we have gained more experience, there are additional challenges that must be overcome to ensure success. These challenges have been overcome in GMP-compliant biobanks.

GMP Ensures that products, consistently produced and controlled, diminishes risks that cannot be controlled by testing of products such as cross contamination and mix-ups and also provides assurance about the quality of the product. Quality-based system is an approach to control collection, processing, storage and release cell therapy or any biologic products. The quality system approach is risk-based and address the following elements: a) facilities (design, access and maintenance) b) equipment (purchase, use and maintenance) c) materials (specifications, purchase, storage and use) d) quality assurance (quality control, validation, qualification and document control).

Manufacturing processes of a biologic product are clearly defined, validated and systematically reviewed to ensure consistency and compliance with specifications and any changes to the process that can impact on the quality of the biologic product are validated under GMP regulation.

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# Effect of Tempol and/or Quercetin in Semen Cryopreservation Medium Improves the Post-Thaw Sperm Function

Tavalaee M[1*], Nasr- Esfahani MH[1,2]

[1]. Department of Reproductive Biotechnology, Reproductive Biomedicine Research Center, Royan Institute for Biotechnology, ACECR, Isfahan, Iran

[2]. Isfahan Fertility and Infertility Center, Isfahan, Iran

Cryopreservation is the most effective method for long-term maintenance of sperm. Despite the success of sperm cryopreservation, this usually used method induces damage on sperm function, viability and, finally decreases semen quality and fertility potential. In addition, percentage of DNA damage increases due to high level of oxidative stress after freeze-thawing. To minimize these damages, we need to increase our insights regarding different cryopreservation procedures, cryoprotectant and antioxidant supplements which can protect sperm membrane during cryopreservation and through these understanding to improve the efficiency of these procedures. Quercetin is a flavonoid with high reactive oxygen species (ROS) scavenging that enhances the activity of antioxidant enzymes and reduces enzymatic activity such as NADPH oxidase and NADH-dependent oxido-reductase. In addition, Tempol, as a superoxide dismutase mimetic agent, converts superoxide to less toxic hydrogen peroxide. We assessed the effect of Quercetin, Tempol, and/or combination of both antioxidants in an optimized commercial cryo-protective media. Therefore, semen samples were cryopreserved in the presence or absence of these antioxidants, and sperm motility, viability, DNA integrity, and level of oxidative stress were compared. The results of current study demonstrated the supplementation of Quercetin or Tempol, but their combination does not improve the quality of cryopreserved human semen. Therefore, the commercially available cryopreservation media supplemented with Quercetin or Tempol could reduce the oxidative stress generated during freeze and thaw, and improve sperm motility, viability, and DNA integrity.

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# Quality Management System of Biobanking

Mahdi Habibi-Anbouhi

Biotechnology Research Center, Venom & Biotherapeutics Molecules Lab., Pasteur Institute of Iran, Tehran, Iran

Many types of biomedical services depend upon the use of high quality human and/or animal biological materials. A biobank is an organized collection of biological materials including: Organs & parts of organs, Tissues (fresh / frozen / FFPE) Cells (mammalian, plant, bacteria, yeast, fungi, archaea, protozoa, algae, parasites, & viruses), Cell extracts, Whole blood and Blood products (plasma, serum, RBC, white cells, buffy coat, protein, etc), Marrow, Fluids (urine, saliva, cerebrospinal fluid, amniotic fluid, synovial fluid, etc), Stool, Proteins and Peptides, DNA & cDNA, and RNA.

Quality Management System (QMS) is a soft infrastructure of biobanks to provide high quality, safely and consistently handled bio-specimens. Supervision of all aspects that influence the quality and safety of biological materials should be considered in the QMS. These aspects include Quality Assurance and Quality Control, Technical staff, Contracted laboratory services, Records management, Facilities, Utilities, Security and Access (staff and visitors), Emergency Preparedness, Storage Equipment and Environments, Equipment Maintenance, Repair and Replacement, Responsibilities, Standard Operating Procedures, Quality Standards, Audits, Safety, Training, Biological Material Tracking, Material Transfer Agreements, Packaging and Shipping, Specimen Collection, Processing and Retrieval, Legal and Ethical Issues for Human Specimens, and etc.

The directors of biobanks should ensure that a Quality Management System is in place to make certain so that the entire operation conforms to the repository's standard operating procedures (SOPs), necessary audits, and government regulations. The Director should require regular, documented, internal reviews or audits to ensure compliance with the SOPs and regulations.

# Effect of Cold Storage of Ovary on Handmade Somatic Cell Nuclear Transfer Outcomes

Mehdi Hajian
Department of Cellular Biotechnology at Cell Science research Center, Royan Institute for Biotechnology, ACECR, Isfahan, Iran.

Over the years, genetic improvement of farm animals has become one of the prime concerns of researchers in the field of assisted reproduction. Artificial insemination, superovulation, in vitro fertilization, embryo vitrification, and transfer have been implemented to reduce reproductive problems or improve offspring derivation from an elite female by reducing generation intervals. Among assisted reproductive technologies, SCNT (somatic cell nuclear transfer) has opened a new fascinating scientific chapter which slowly but gradually is gaining its place. Handmade cloning (HMC) as a new technique, in SCNT can be used to produce a large number of embryos in each run. Another problem faced in SCNT laboratories, is the arrival time of ovaries from slaughterhouses or time taken for ovaries to reach the SCNT lab. Therefore, maintenance of ovary at low temperature or cold storage of ovaries has been recommended. In this regard, we pursued main aims in this study assessing the role of storage conditions for ovaries on pre-implantation development and freeze ability of derived blastocysts. For this purpose, recovered oocytes from warm storage ovaries and cold storage ovaries were used to produce SCNT embryos. There was no difference between the cleavage (95.91±2.93 vs. 95.68±1.67) and blastocyst formation (10.94±0.9 % vs. 10.69±1.17 %) rates of recovered oocytes from warm and cold storage ovaries, respectively. The re-expansion rate of vitrified blastocysts was significantly higher in warm storage ovaries (90±11.26 %) than clod storage ovaries (66.3±8.7 %). In conclusion, the results of this study showed the similar *in vitro* pre-implantation developmental potentials of warm and cold storage ovaries.

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# Plant Cryopreservation

Elyas Aryakia

Plant Bank, Iranian Biological Resource Center (IBRC) (ACECR), Karaj, I.R. Iran.

Two conservation strategies, *in situ* (conservation of plants ecosystems and natural habitats) and *ex situ* (conservation of plants outside their natural habitats), are employed for conservation of plant genetic resources. Cryopreservation, as one of the *ex situ* plant germplasm conservations, is the only technique currently available to ensure the safe and cost-efficient long-term conservation. This technique is based on tissue storage (meristems, seeds, dormant buds, zygotic and somatic embryos or pollen but also callus cultures and cell suspensions) at ultra-low temperature of liquid nitrogen (LN). At this temperature (−196°C) the biological activities and cell division are stopped, allowing for long-term conservation. Common procedures for plant cryopreservation include the following successive steps: pre-growth of plants, cryoprotection of plant materials, slow cooling, and rapid immersion in LN, storage, rapid thawing, and recovery. To date, cryopreservation techniques have been successfully applied to a large number of plant species including woody and herbaceous plant species ranging from temperate to tropical regions. Our results of cryopreservation treatment on orthodox seeds showed that there are significant differences among plant species for all germination traits. Moreover, in comparison between cryopreservation and control treatments in each species, no significant difference was observed. So, we can emphasis cryopreservation as a suitable, alternative and inexpensive method for healthy long term storage of orthodox seed in germplasm resources conservation centers. The main advantage of this technique is the reduction of *in vitro* culture costs, required space, contamination and somaclonal variation risk. By using cryopreservation technique, we can provide a conservation method of which cost is %1 of *In situ* plant germplasm conservation. Recently, cryopreservation has been used for cryotherapy, i.e. a brief treatment of shoot tips in LN. In cryotherapy, plant pathogens such as viruses and bacteria are eradicated from meristems. Moreover, research is actively performed to improve knowledge of biological mechanisms underlying the tolerance of plant tissues to desiccation and cryopreservation.

The prevalence numbers of childhood cancer survivors have increased today because of timely diagnosis and new treatments. Increased awareness about the impact of various cytotoxic treatments on ovary has now resulted in a surge in the number of patients seeking help to preserve their fertility. Cryopreservation of embryos is a standard technique for fertility preservation when there is adequate time for ovarian stimulation. If patients have no partner or unwilling to use donor sperm, oocytes can be frozen instead. At present, only fertility preservation option that can be offered to prepubertal girl and women who have limited time for treatment is ovary cryopreservation. Current experience about ovary cryopreservation and transplantation is limited.

From December 2000 until 2010, the researchers at Royan Institute conducted a wide range of investigations on ovarian tissue cryopreservation with the intent to provide fertility preservation option to cancer patients that were considered to be candidates for these services. In 2010, Royan institute established the Royan Human Ovarian Tissue Bank as a subgroup of the embryology department. Since its inception, approximately 500 patients between the ages of 7- 47 years have undergone consultations. Ovarian samples were cryopreserved from 74 patients right now, (age: 7-35 years) diagnosed with cervical adenocarcinoma (n=9); breast carcinoma (n=7), Ewing's sarcoma (n=7), ovarian tumor (n=7), endometrial adenocarcinoma (n=4), malignant colon tumors (n=3), as well as Hodgkin's lymphoma, major thalassemia and acute lymphoblastic leukemia (n=1-2 patients for each disease). Additionally, two patients requested ovarian tissue transplantation after completion of their treatments.

Our main goal is to preserve ovarian tissue by using the best cryopreservation protocol and also tries to set up the in vitro culture of human follicle and investigate different molecular mechanisms which are involved in these procedures to improve follicle development.

**Image processing of ovarian tissue cryopreservation**

در هر تولد، حیاتی دیگر نهفته است

## شرکت فناوری بن یاخته های رویان

# بانک سلولهای بنیادی خون بند ناف

**آیا می دانید؟**

**پیوند سلول های بنیادی خون بند ناف در درمان افراد مبتلا به بیماری های خونی مانند: سرطان های خون، لنفوم، تالاسمی و ... نقش بسزایی دارد.**


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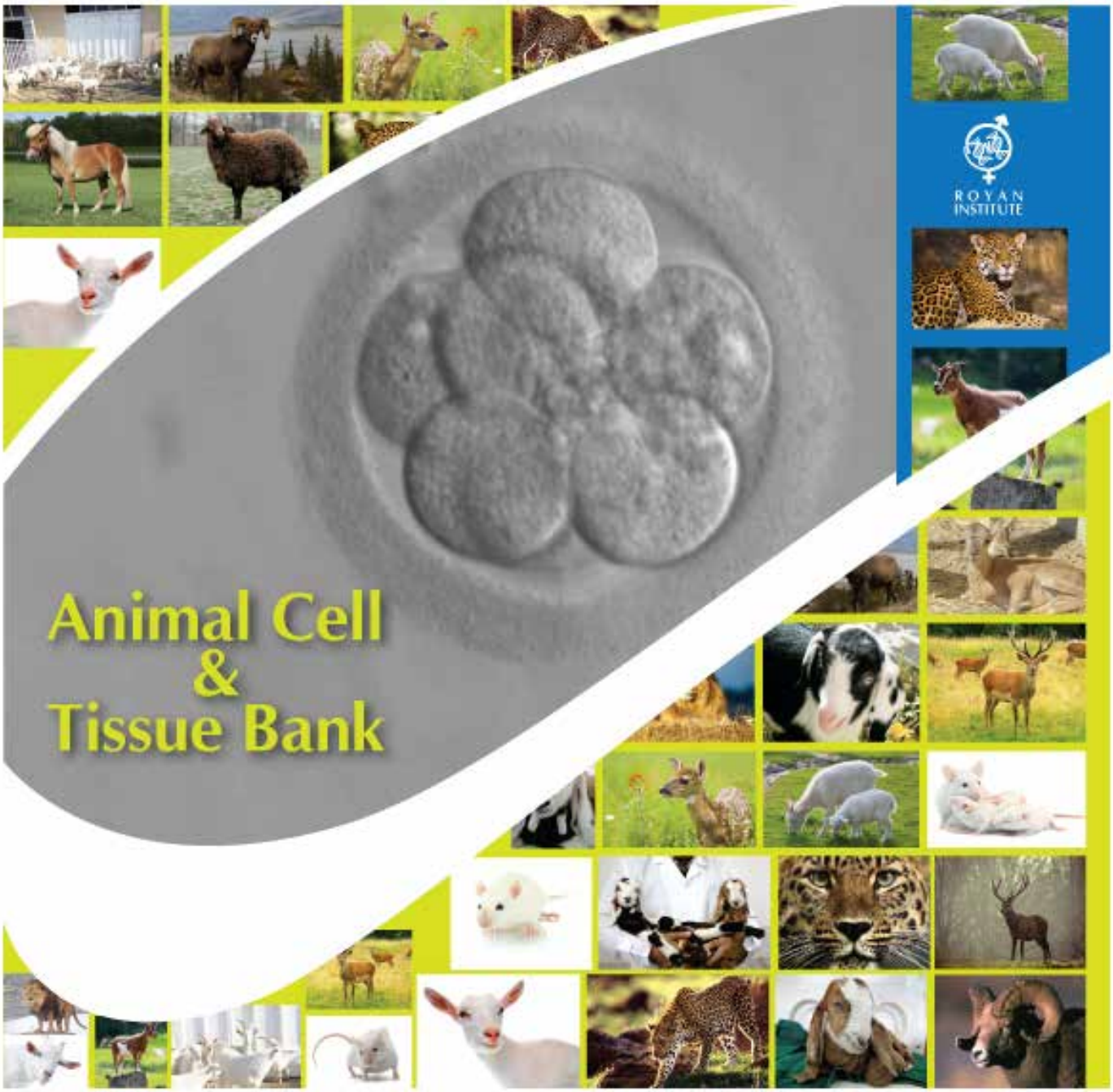
# Animal Cell and Tissue Bank

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# DNA Bank in Reproductive Genetic Department, Royan Institute

Diagnosis of genetic disorders and basic research on human genes require accessibility to the Human Genome. Sampling collection is one of the basic parts of an investigation. Genetic reserves are as one of the precious sources of any country. Keeping the genome samples as DNA bank is secure and long term storage of an individual's genetic material. Researchers around the country are able to access the database and submit requests for genome material of different abnormalities through collaboration with this kind DNA Bank. . DNA is commonly extracted from blood, but can also be obtained from cheek cells, saliva, or other tissues. To ensure that genetic material is available and that biodiversity resources remain within the institute, Royan DNA Bank was founded in 1384 (2006), proudly to be the first DNA sample collection from all kind of infertility disorders in the country.

One of the most beneficial reasons for having the DNA bank is that the biodiversity resource can be retained nationally and be easily accessible to researchers. Banking of the genetic resources also provides a way to ensure that samples are available from a wide variety of ethnicity and different infertility causes, a strategy especially important when DNA samples collection could be difficult to be saved depending on the patient symptoms and the type of disorder, meanwhile, it enables the researchers to carry out the projects more efficient and quickly.

Genomic samples preserved in DNA banks will be stored within extracted from cells and purified before storage. The quality of the DNA is expressed through purity. Careful and clean extraction methods are critical during processing. DNA banks should determine who is the collector or the curator and responsible for extracting and storage as part of the quality control process. Once extracted DNA is a stable biomolecule, DNA is better conserved in a form that is close to the original state and most DNA banks store cells or tissues and extract DNA upon request. DNA samples can be maintained at -20ºC for short- and mid-term storage (up to 2 years), and at -70ºC or in liquid nitrogen for longer periods.

Royan DNA Bank comprises approximately 5500 DNA samples and holds samples obtained from infertility patients, associated with full relevant documentation (the informed consent, the provenance, the extraction method and date, and the year of incorporation into the DNA bank).

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