Translation of cryobiological techniques to socially-economically deprived populations: Part 1: Cryogenic preservation strategies

Buriak, Iryna
Institute for Problems of Cryobiology and Cryomedicine of the National Academy of Sciences of Ukraine
23, Pereyaslavska str., 61016, Kharkiv, Ukraine

Fleck, Roland
Centre for Ultrastructural Imaging, Kings College London
New Hunts House, Guy’s Campus, London SE1 1UL, UK

Goltsiev, Anatoliy
Institute for Problems of Cryobiology and Cryomedicine of the National Academy of Sciences of Ukraine
23, Pereyaslavska str., 61016, Kharkiv, Ukraine

Shevchenko, Nadiya
Institute for Problems of Cryobiology and Cryomedicine of the National Academy of Sciences of Ukraine
23, Pereyaslavska str., 61016, Kharkiv, Ukraine

Petrushko, Maryna
Institute for Problems of Cryobiology and Cryomedicine of the National Academy of Sciences of Ukraine
23, Pereyaslavska str., 61016, Kharkiv, Ukraine

Yurchuk, Taisiia
Institute for Problems of Cryobiology and Cryomedicine of the National Academy of Sciences of Ukraine
23, Pereyaslavska str., 61016, Kharkiv, Ukraine

Puhovkin, Anton
Institute for Problems of Cryobiology and Cryomedicine of the National Academy of Sciences of Ukraine
23, Pereyaslavska str., 61016, Kharkiv, Ukraine
Rozanova, Svitlana  
Institute for Problems of Cryobiology and Cryomedicine of the National Academy of Sciences of Ukraine  
23, Pereyaslavska str., 61016, Kharkiv, Ukraine

Guibert, Edgardo Elvio  
Centro Binacional (Argentina-Italia) de Investigaciones en Criobiología Clínica y Aplicada, Universidad Nacional de Rosario, Rosario, Argentina and Consejo Nacional de Investigaciones Científicas y Técnicas  
Avda. Arijon 28BIS (2000), Rosario, Argentina

Robert, Maria Celeste  
Centro Binacional (Argentina-Italia) de Investigaciones en Criobiología Clínica y Aplicada, Universidad Nacional de Rosario, Rosario, Argentina and Consejo Nacional de Investigaciones Científicas y Técnicas  
Avda. Arijon 28BIS (2000), Rosario, Argentina

de Paz, Leonardo Juan  
Centro Binacional (Argentina-Italia) de Investigaciones en Criobiología Clínica y Aplicada, Universidad Nacional de Rosario, Rosario, Argentina and Consejo Nacional de Investigaciones Científicas y Técnicas  
Avda. Arijon 28BIS (2000), Rosario, Argentina

Powell-Palm, Matthew J.  
Department of Mechanical Engineering, University of California Berkeley  
6124 Etcheverry Hall, Hearst Ave, Berkeley, CA, 94720

Fuller, Barry ¹  
Division of Surgery & Interventional Science, UCL Medical School, Royal Free Hospital  
London NW3 2QG, UK

ABSTRACT

Use of cold for preservation of biological materials, avoidance of food spoilage and to manage a variety of medical conditions has been known for centuries. The cryobiological science justified these applications in the 1960s increasing their use in expanding global activities. However, the engineering and technological aspects associated with cryobiology can be expensive and this raises questions about the abilities of resource-restricted low and middle income countries (LMICs) to benefit from the advances. This review

¹ Address for correspondence – b.fuller@ucl.ac.uk
was undertaken to understand where or how access to cryobiological advances currently exist and the constraints on their usage. The subject areas investigated were based on themes which commonly appear in the journal Cryobiology. This led in the final analysis to separating the review into two parts, with the first part dealing with cold applied for biopreservation of living cells and tissues in science, health care and agriculture, and the second part dealing with cold destruction of tissues in medicine. The limitations of the approaches used are recognised, but as a first attempt to address these topics surrounding access to cryobiology in LMICs, the review should pave the way for future more subject-specific assessments of the true global uptake of the benefits of cryobiology.

Keywords: cryobiology, preservation, cells, developing countries, low income countries, middle income, banking, blood, plants, seeds, cord blood, animal germplasm, reproductive medicine, tissue banking, new approaches.

1. INTRODUCTION

The ability to use low temperatures for preventing spoilage of foods and crops has provided significant benefits to human populations since the dawn of civilization and many historical texts have been produced on this topic, such as Boyle’s treatise from the 17th century [1]. Equally, freezing in the natural environment has been a recurrent and often spectacular event with in many cases significant human impact and a source of scientific research in situations such as the early polar expeditions [2]. However, it was not until the middle years of the 20th century that science found ways to merge freezing to extreme low temperatures and associated medical therapies with the foundation of a new scientific discipline, termed cryobiology. Two major pathways were involved; for one, cryogenic freeze-preservation or cryopreservation of living cells was first achieved for sperm by Polge and colleagues [3] in which the recovered cells
could be returned to normal vitality after thawing (recently reviewed by Pegg [4]); in the second pathway, controlled cryogenic freezing could be applied to destroy pathological tissue such as tumors during patient treatment [4] by cryosurgery (recently reviewed by Baust and colleagues [5]). Both disciplines were combined in cryobiology and depended upon the growing scientific understanding of the physical changes of water during freezing, the status of different aqueous systems when ice nucleates and bio- materials are transferred below −100°C, and how different solutes can interact in the biophysics of freezing to either enhance recovery of living cells or in contrast to enhance cell killing if that is the desired outcome. More recently, an important 3rd application for cryobiology in the extreme high resolution ultrastructure of biomaterials in cryogenic electron microscopy has been developed. This will be beyond the scope of the current review, but has been recently reviewed by Danev [6].

From the early 1970s there has been a growing impact of translational cryobiology across many areas of healthcare, species management and conservation, and biotechnology. Cryogenic applications have become embedded in so many current technologies that they have become routine and second nature. However, this does require access to reliable sources of cryogens (most often liquid nitrogen with its’ advantageous properties as a non-flammable agent with a boiling temperature of −196°C), equipment capable of operating in these temperature ranges, skilled personnel, and a holistic understanding of fundamental cryobiology. These all come with associated costs, which can often only be met within economically privileged societies. For example, as only one case, in the UK capital London, cryobiology has
multiple sites dealing with applications in cryopreservation of reproductive cells, blood cells, stem cells, novel autologous cell therapies, clinical tissue banks, plant seed banks, cryogenic imaging centers and specialist cryosurgeries (B. Fuller, personal observation). This is repeated in centers across the USA, Europe and the Pacific Rim too numerous to identify individually. In contrast, the purpose of our current review is to look more closely at how the benefits afforded by cryogenic applications may or may not be spread at present on a global basis, where many countries are economically stressed with only middle or low income economies. This will be the first opportunity to do this as far as we are aware, and will inevitably be flawed by the limited data availability. However, we believe that this can serve as a ‘first-pass’ attempt which can highlight topics and stimulate further research and translation by those working in cryogenic applications and cryobiology, to rise to the challenges of truly global technology transfer.

2. METHODS

This review has been constructed to a very large degree under the good offices of the UNESCO Chair in Cryobiology. This UNESCO Chair was established in 1998 by invitation from UNESCO central offices to the then Director of the Institute for Problems of Cryobiology and Cryomedicine of the Ukraine Academy of Sciences in Kharkiv (IPC&C), Professor Valentin Grischenko and till now is hosted by the Institute (Ukraine). In 2019 the Chair joined the UNITWIN Network in Life Sciences to extend the activities on research and postgraduate education in biophysics, biotechnology and environmental health. The main field of expertise of this Chair is cryobiology and
cryomedicine. The Chair basic objectives are listed as to promote the application of cryobiological science and technology with a view to improve quality of life, particularly in developing countries; to provide training in the field of cryobiology to the next generation of science and engineering graduates from developing countries; to foster exchange of information at international level and to establish an information bank in order to reduce the gap between the interested institutions and centers. One of the goals of the Chair was to provide information and training in cryobiology to anybody requesting this, as an academic philosophy and largely by using the worldwide web. It was recognized that cryobiology has a truly global spread of applications with large potential benefits to science and society, across the complete economic scale from low to high income countries. However, the uptake of the different activities within cryobiology will of course be determined by the local economic environments and the relevant costs, such that not all countries can access all of the technologies, or can access them only in a fragmented fashion. Thus in order to address the questions set by this review, the UNESCO Chair in Cryobiology was able to call upon established global links to interrogate the questions set out. Current information on the UNESCO Chair in Cryobiology can be found at http://cryo.org.ua/ipk_eng/unesco.html and is under the leadership of the current Director of IPC&C in Kharkiv, Professor Anatoliy Goltsev. Where gaps were identified in the review and could not be answered from the UNESCO Chair contacts, in two topic areas, information has been gathered by workers based with a globally-recognized center of excellence in cryobiology, University of California at Berkeley, USA.
Individuals used well-understood information sources such as Library of Congress, WHO reports, FAO reports, World Bank Data, Google Scholar, Research Gate, PubMed data bases to identify publications in their topic areas, and freely-available national or international reports focusing on the low to middle income nations, often which included commercial activities. This information is clearly skewed towards academic activities, but since there has not been any similar publication on the questions posed by our review, this can be seen as a first attempt to collate the data, and to inform future debates about how best to deliver the benefits from translatable cryobiology to global societies on a more equitable basis.

Translational cryobiology is applied in many biotechnological, clinical and laboratory processes too numerous to interrogate in the scope of this review. However, there are a number of applications, based on their repeated appearances in meetings of specialized societies such as Society for Cryobiology and journals such as Cryobiology, which can be taken as broad representatives of cryobiology. For the purposes of this review, these topics can be listed as:

- Cryotechnologies for Blood Banking
- Cryobanking of Plant Resources
- Cryopreservation of Animal Germplasm
- Cryopreservation in Human Reproductive Medicine
- Cryopreservation of Cord Blood
- Medical Tissue Banking within applied cryobiology
Additionally, the development of novel technologies for applied cryobiology continues at a fast pace. These should be discussed in the context of how easily or how frequently these are likely to be taken up in socio-economically deprived regions. Thus the following section has been included:

- New approaches in cryobiology

Lastly, the power of cryogenic cooling and induction of ice nucleation can be harnessed not only for preservation, but, with the correct scientific understanding, for selective bio-destruction. The most-widely used of such applications is for cryo-destruction of pathological tissues such as tumors in the field of Cryosurgery. This conceptually different topic is presented in Part 2 of this review as – PART 2 Cryosurgery.

3. GLOBAL APPLICATIONS OF CRYOBIOLOGY

3.1 CRYOTECHNOLOGIES FOR BLOOD BANKING

If any material that is included in a paper is under copyright, the authors of the paper are responsible for obtaining copyright permissions, including any information required by the copyright holder, and be able to produce such permissions upon request. Blood banking was one of the first translational applications of cryobiology from the middle of the 20th century. The technologies span both refrigerated storage and cryopreservation. Blood is a life-saving resource of strategic importance. The key problems of adequate blood supply to world’s population are blood safety and availability [7, 8]. As reported by World Health Organization (WHO), availability, safety
and using of donor blood and its products differ greatly depending on the development level of countries [7, 8]. According to current World Bank Classification [9, 10], there are 81 countries with high-income economies (GNI per capita ≥$12,056), 56 upper-middle income countries ($3,896–12,055), 47 lower-middle income ($996–3,895) and 34 low-income countries (≤$995).

The availability of blood is assessed as number of blood donations per 1000 people. The rates of availability are 4.4 in low-income, 8.1 in lower-middle income, 15.1 in upper-middle income and 32.6 in high-income countries [8]. Moreover, 42% of all (117.4 million) blood donations are collected in high-income countries, where only 16% of world’s population live [8]. Limited blood availability in low- and middle-income countries is caused by deficiency in regular non-remunerated donors, donor sickness, lack in kits for screening, discarded donations due to bloodborne infections [8, 11-14]. There are also cultural limitations for voluntary blood donations [11, 15]. Thus, the blood resources in low- and lower-middle income countries are significantly restricted.

Blood banking systems are necessary to solve the problems of timely and sufficient blood providing. The WHO recommends storing whole blood and the red cells at the temperatures of +2...+6 °C [16]. However, hypothermic storage limits the shelf-life of whole blood and its components and storage additives can significantly affect recipient’s metabolome [17-19]. Cryopreservation and low-temperature storage of blood and its components in blood banks allow providing high-quality standards, reliable testing for transfusion-transmitted infections (TTI), sufficient reserve and appropriate use of donor blood in routine medical practice and emergencies [13, 20].
On the example of India the rural areas of developing countries have been shown to be isolated from blood banks [15]. Frozen blood can be available when there is no access to refrigerated fresh donor blood. Moreover, cryopreservation prolongs the shelf-life of blood cells for transfusions [13, 17, 21]. Strategic reserve of frozen blood can improve logistics in blood supply and provide long-term storage of excessed blood units, which are usually wasted [13]. It is necessary for appropriate use of blood stock in developing countries.

Transfusions of autologous blood cryopreserved before surgical care enables reduction of TTI and immunosuppression as compared to allogenic transfusions [21]. Studies in Nigeria evidenced that autologous blood transfusions were safe and cost effective [11].

Cryopreservation of blood and its components was applied for the decades mainly in military medicine, in blood crisis situations as well as for rare and autologous blood storage [17, 21-23].

Organization of blood cryobanks requires financial investments, specially trained personnel and national regulations. Blood cryopreservation was always an alternative to hypothermic blood storage and it was not routine because of its apparently high cost. Indeed, cryopreserved blood cells seem to be more expensive than hypothermically stored due to prolonged storage time. However, the economical difference between both storage methods is probably overestimated, because the costs of treating and managing adverse events of refrigerated blood cells should be also taken into account [21]. In any case, the benefits of blood cryobanks are expected to exceed their costs.
High-income countries can contribute to solving blood biobanking problems in low- and lower-middle income countries. Four Dutch portable military blood cryobanks were organized in Afghanistan in 2006–2016. Most of transfusions in 2006–2010 were given to Afghan civilians [24]. Australian Defense Forces in collaboration with Australian Red Cross started implementing frozen blood supply to civilians and militaries worldwide [24, 25].

Researchers understand the importance of cryopreservation for providing developing countries with blood. The efforts to make such technologies more accessible are known. Sen et al. have shown successful automated cryopreservation of donor blood with glycerol at −80°C and proposed this method for establishment of frozen blood bank in India [13]. Although frozen red cells are usually stored at −80°C, the method of freezing down to −20°C under protection of PVA and HES was proposed [26]. This approach allows using minimal electricity even from solar batteries or portable generators [26]. It may be applicable in low-income countries.

Currently National blood cryobanking is not developed in low- and lower-middle income countries. Ukraine stands out against the general background of these countries. The Institute for Problems of Cryobiology and Cryomedicine (IPC&C) works since 1972 in Ukraine [27]. There is a licensed Low Temperature Bank for long-term storage of different cells and tissues. This bank is a part of Interdepartmental Scientific Center (ISC) of Cryobiology and Cryomedicine of the National Academy of Sciences of Ukraine, Academy of Medical Sciences and Ministry of Health Care of Ukraine, founded
in 1997 [27]. The Low Temperature Bank has the status of the national endowment of Ukraine [28].

The effective methods of blood cells cryopreservation and special cryobiological equipment were developed in the IPC&C [29-32]. Extensive experience in the development of various cryotechnologies has been accumulated there. These technologies could be shared among other countries.

In conclusion, cryopreservation of blood cells for creating strategic reserve in lower-medium- and low-income countries is very prospective. It can improve blood safety and availability in these developing countries. Hence, the mortality from anemias, massive hemorrhagies and transfusion-transmitted infections can be reduced.

3.2 CRYOBANKING OF PLANT RESOURCES

Plants play an important role in ecosystems in which all animals, including humans, live, survive and grow. We rely on plants for many aspects of life for example medicine, food and firewood. Furthermore, plant species may have additional uses which have not yet been uncovered. Humans have a moral responsibility to conserve biodiversity for future generations. Storing orthodox seeds is an efficient way of conserving individual plant species because limited space is required and such seeds can be stored easily for long periods of time. In addition, seed collections represent an extremely useful research resource. There are more than 1750 seed banks in the world, the majority of which conserve crop diversity [33].
One of the fundamental principles of preserving germplasm is that the physical parameters of the premises of the gene bank in which it is stored must meet the standards for protecting samples from any external factors, including natural disasters and human exposure. Relevant safety systems are needed to ensure that refrigeration equipment, spare generators and gene bank equipment to monitor power outages are in good working condition, and existing monitoring devices allow you to monitor the most important parameters over time.

Since cryogenic storage requires liquid nitrogen, supplies of a cryogenic substance must always be established. It is very important to maintain the required level of liquid nitrogen, regardless of whether it is manually or automatically.

Some of the poorest countries in the world are the richest in terms of the agro-diversity. Many traditional varieties have been lost and many of those that remain can now be found only in gene banks.

After studying the reports on the state of plant genetic resources for FAO and official sites of plant genetic resources centers among 147 developing countries, we can do the following conclusions. Almost all countries have a collection of plant genetic resources, however gene banks in which seeds are stored at sub-zero temperatures are among the countries that belong to low-income economies: Malawi, Mali, Uganda, Ethiopia, Niger [34], Nepal [35], Sudan, Yemen, Zimbabwe [34], Burkina Faso; lower-middle-income economies: Bolivia, Bhutan, Indonesia, Egypt, Ghana, El Salvador, India [36], Ivory Coast, Kenya [34], Morocco, Nigeria, Pakistan, Philippines, Ukraine, Zambia; upper-middle-income economies: Albania, Armenia, Azerbaijan, Brazil [37], Bulgaria,
China [38], Colombia, Costa Rica, Cuba, Ecuador, Fiji, Jordan, Lebanon, Macedonia, Malaysia, Montenegro, Russian [39], Turkey [40], Romania, Surinam, Venezuela [41]. Some countries reported about cryopreservation of plant germplasm at liquid nitrogen temperatures [41]: Indonesia, Bolivia, Ghana, India, Pakistan, Philippines, Fiji, Costa Rica, Cuba, Malaysia, Surinam, Nepal, Ecuador, Egypt, Russia, Kazakhstan [42].

However, when we studied the international database [43] only 25 of these countries have submitted this information and no one reported about cryopreservation [44].

Samples of seeds that are stored at low temperatures are available by link https://www.genesys-pgr.org/explore/map?filter=%7B%7D [43].

Almost all developing countries except Kosovo, Marshall Islands, Cabo Verde, Qatar (these countries do not have information on plant genetic resources) store their seed collections in low-temperatures gene banks of high-income countries. Crops and countries that have low-temperature banks are available from Genesis [45].

In addition to crops, many seeds of wild species are stored in low-temperature banks.

Information from Botanic Gardens Conservation International's databases indicates that there are at least 350 seed banking botanic gardens in 74 countries. Seed banking involves collecting seeds from wild plants, drying and storing them in cool conditions [46]. Most of developing countries store the wild plant species seeds into liquid nitrogen in Royal Botanic Gardens Kew's Millennium Seed Bank in the United Kingdom.
National Plant Gene Bank of Ukraine based in the Plant Production Institute nd.a. V.Ya. Yuryev NAAS (Kharkiv) stores more than 32 thousand samples at low temperature of −20°C. Scientists of the IPC&C developed methods of cryopreservation of agricultural crops seeds; meristems of potatoes, grapes, garlic [47-49]. Methods of cryopreservation of sweet potato, tomato meristems and cuttings of grapes are under development now in the Laboratory of Phytocryobiology which was found in 2016 [50, 51].

In conclusion, the development of low-temperature seed banks in low-income countries requires the involvement of expensive equipment that can only be purchased with sponsorship funds. For the better functioning of such banks, it is necessary to coordinate their work.

3.3 CRYOPRESERVATION OF ANIMAL GERMPLASM

*Ex situ in vitro* preservation means preservation under cryogenic conditions including interalia, the cryopreservation of embryos, semen, oocytes, somatic cells or tissues having the potential to reconstitute live animals at a later date [52, 53].

According to FAO data in 2007 only 37% of country reports indicate the presence of *in vitro* preservation (Table 3.3.1), but in 2015 almost a half (45%) of reporting countries indicate that they have an operational *in vitro* gene bank for animal genetic resources. A further third part of reports informed that they have plans to develop the banks.

The percentage of countries that reported having *ex situ in vitro* preservation programs was higher than those that reported having gene banks. The discrepancy is
accounted for mainly by the fact that some countries that do not have gene banks report the storage of cryopreserved genetic material for use in research or breeding programs or for preservation purposes within the framework of small-scale projects [52, 53].

Semen is by far the most commonly stored material, followed by embryos. However, isolated DNA, somatic cells and oocytes are stored in a substantial number of gene banks. There is some regional variation. For example, more than half of African countries reporting the presence of a gene bank indicate that they don’t store material other than semen. The use of gene banks to store material from breeds that are not currently regarded as being at risk of extinction is quite widespread.

Semen is preserved from all the main species, and embryos of cattle, sheep and goats are also stored. Only a few gene banks store poultry and horse semen. In developed countries, there is strong collaboration between gene banks and the animal breeding industry and breeders’ associations with respect to the collection of genetic material. In developing countries that implement in vitro preservation measures, activities are limited to storage of semen from some local cattle and sheep breeds at private or governmental institutions.

Only a minority of country reports indicating the presence of a gene bank state those livestock keepers or breeders’ associations are involved in its operation.

The breed coverage of ex situ in vitro conservation programs is still very limited overall, and many countries have no gene banks. Many report that they have plans to establish gene banks, but lack of funding and lack of technical skills often remain
significant constraints. Collaboration at regional or subregional level is a potential means of avoiding duplication in the use of resources, provided the relevant institutional and legal arrangements can be put in place. Interest in initiatives of this kind is reported from several regions and subregions.

Africa

*In vitro* preservation is not widespread in Africa. The majority of countries report that they have no gene bank and the proportion of breeds covered is low. The knowledge necessary to implement such programs is scarce, and the required infrastructure (e.g. liquid nitrogen facilities) is not available, or cannot be adequately maintained. *In vitro* activities are limited to the storage of semen from some local cattle breeds at private or governmental institutions. Some countries also mention the storage of semen from imported exotic breeds as a strategic activity.

However, several country reports mention plans to establish subregional gene banks in Africa. The report from Uganda, for example, mentions the objective of developing a gene bank in collaboration with Burundi, Kenya, Rwanda, South Sudan and the United Republic of Tanzania (mostly LIEC). The report from Togo mentions plans to collaborate with other countries of the Economic and Monetary Union of West Africa to create a regional bank or strengthen the capacity of the gene bank of the International Centre of Research and Development of Livestock in the Subhumid Zone, based in Burkina Faso (LIEC). The report from South Africa (UMIE) mentions the intention to collaborate with other Southern African Development Community countries Botswana, Namibia (both UIEC), Zambia (LMIEC), Mozambique and Zimbabwe (both LIEC) [52, 53].
Asia

More than half (60 percent) of country reports from Asia indicate the presence of a gene bank. However, there are substantial differences between the subregions (Table 3.3.1). In general, the gene banks in East and South-East Asia are more developed than those in the other two subregions.

East Asia has a higher proportion of its chicken breeds stored in gene banks than any other subregion or region in the world. This is mainly a result of the presence of well-developed gene banks in China and Japan. Semen is preserved from all the main species, and embryos from cattle, sheep and goats are also stored. In a few countries (e.g. Japan) tissue DNA is collected from all the main species. Governments undertake these in vitro activities in collaboration with industry.

Although gene banks are relatively uncommon in the reporting countries of Central and South Asia, some countries from these subregions report well-developed gene banks. The gene bank of the Islamic Republic of Iran (UMIEC), for example, includes genetic material in the form of semen, embryos, oocytes and isolated DNA from cattle, sheep, goats, horses, buffaloes, Bactrian camels and dromedaries. Material from the gene bank has been used to introduce genetic variability into in situ and ex situ populations. The gene bank of India (LMIEC) includes semen and isolated DNA from cattle, sheep, goats, buffaloes, horses and asses. Cattle genetic material from the gene bank has been used to increase the genetic variability and population sizes of cattle breeds such as the Tharparkar, Sahiwal, Krishna Valley and Haryana. In Southeast Asia, Malaysia, and Thailand (both UMIEC), the Philippines, and Vietnam (both LMIEC) all
report the presence of a gene bank, while Indonesia (LMIEC) reports plans to develop one. These gene banks are used mainly for introducing genetic variability into breeding programs involving \textit{ex situ} populations. With regard to international collaboration in gene banking within the region, the country report from the Philippines mentions plans for collaboration between India, Pakistan and the Philippines in the \textit{ex situ in vitro} preservation of buffaloes [52, 53].

\textbf{Europe and the Caucasus}

The majority of the countries in the region report well-established gene banks. In many cases this is restricted to the storage of semen from a limited number of cattle and sheep breeds. A few countries (the Nordic countries, France, the Netherlands, Poland, the Czech Republic and Hungary) have gene banks preserving semen from the main species. In some cases, embryos of cattle, sheep and pigs are also preserved, and in a few countries, cattle oocytes or tissue DNA are stored. These banks are recently founded or are under construction. A strong collaboration with the animal breeding industry exists in most countries. The gene banks need to be further developed – with respect, for example, to ownership and access, information and documentation, and optimization of the core collection and the ratio between gametes and embryos. Despite the presence of a rich AnGR diversity in combination with real threats (such as political instability) \textit{in vitro} preservation programs are largely absent in the eastern parts of the region, with the exception of Ukraine [52].

\textbf{Latin America and the Caribbean}
In this region, the number of countries with active preservation programs is low, although many countries report a very rich national biodiversity.

*In vitro* preservation is limited to the storage of semen and sometimes also of embryos from a few breeds. The initiatives for establishing cryobanks are mainly taken by governments with help from universities and institutes. Brazil is the first country in this region to have established a gene bank [52, 53].

**North America**

In the United States of America and Canada, animal generic resources are seen as a strategic resource for national food security, which may be threatened by bioterrorism. This is one of the reasons why the United States of America invested in the establishment of an *in vitro* preservation program and a gene bank. Collections are being built up very quickly, in close collaboration with the industry [52]. Breeding companies use the gene bank as a back up of their breeding work. In Canada, a program for *in vitro* preservation has been developed and will be implemented in the near future. There will be close collaboration between the United States of America and Canada in gene bank activities. They share information and documentation programs, and are discussing taking care of each other’s back-up *in vitro* collections [52].

**Southwest Pacific**

In general, governments in this region show little awareness of the strategic value of the genetic diversity of livestock [52]. Gene banks are present only in Australia and New Zealand. In both countries, the banks are operated by private bodies rather
than by the public sector. In New Zealand, the Rare Breeds Preservation Society of New Zealand, in collaboration with a private cryostorage facility, maintains a genetic repository at which genetic material from at-risk breeds is stored in the form of semen and embryos. The gene bank operates entirely on the basis of private funding. No information was provided in the country report about the number of breeds from which material is stored. A similar approach is taken in Australia, where breeding organizations and civil society organizations support ex situ preservation. In vitro programs in Australia only include at-risk breeds with commercial potential. There are no gene banks in the small island countries of the region [52, 53].

**Near and Middle East**

Oman is also the only country in the region that reports a gene bank (semen and isolated DNA of two multi-purpose cattle breeds are stored and are used for both preservation and breeding purposes) [53].

**Post-Soviet countries**

**Russian Federation**

A cryobank of feline genetic resources has been created in Novosibirsk at the Institute of cytology and genetics SB RAS [54]. In addition, an Experimental Genetic Cryobank has been created at the Institute of Cell Biology RAS. The main provisions of the “Program for the preservation of genetic resources using cryopreservation methods” were developed by Prof. Veprintsev back in 1978. The cryobank contains a collection of animal biomaterials: sperm of several marine invertebrate species (sea urchins, sea cucumbers) and amphibians, several fish species, including rare, 3 rare bird
species (Siberian Crane, Dahurian crane, Japanese crane), more than 20 species of mammals, many of which are endangered (for example, Japanese macaque, markhor, goat, bison, northern fur seal, Amur tiger, Far Eastern leopard) [55].

Ukraine

Sperm of valuable commercial, rare and endangered species of fish are stored in Low Temperature Bank of the IPC&C. The cryopreserved samples are extremely valuable and some of them are stored for more than 30 years. The bank stores frozen sperm of species that are endangered, e.g. aral thorn or ship Acipenser nudiventris Lovetzky, 1828, Huso huso Linne, 1758, from Azov Sea, green sturgeon (Acipenser medirostris Ayres, 1854) from the Far East, stellate sturgeon (Acipenser stellatus Pallas, 1771), sterlet (Acipenser ruthenus Linne 1758) from the Caspian Sea, Russian sturgeon (Acipenser guldenstadtii colchicus Brandt., 1833) sperm from Berdyansk, troepera (Tripterygion tripteronotus Russo, 1810), species from the Black Sea [56].

3.4 CRYOPRESERVATION IN HUMAN REPRODUCTIVE MEDICINE

The technology of reproductive cells and embryos cryopreservation in most countries is an integral part of ART and is regulated by law, and legal regimes may be restrictive, permissive and uncertain. The use of cryopreservation of reproductive cells and embryos in low-income countries depend on the level of assisted reproductive technologies development and religious beliefs.

In all Muslim countries surveyed in the mid-1990s, including the countries of the Middle East, such as Egypt, Iran, Kuwait, Jordan, Lebanon, Morocco and Turkey, as well
as a number of Muslim countries that are not part of the Middle East, including Indonesia Malaysia and Pakistan – IVF sperm donation and all other forms of gamete donation are strictly prohibited. Therefore, in the listed countries, reproductive cell cryobanks for donation programs are prohibited [57].

Cryopreservation in itself does not entail violations of Islamic law, but scientists warn that frozen embryos are the exclusive property of the couple and can be transferred only to the same wife in a sequential cycle limited during the term of the marriage contract. In other words, storing the husband’s sperm to fertilize the wife in the event of his death is illegal. According to Islamic law, death terminates the marriage contract, and the widow can freely remarry after a mandatory waiting period (al-‘Idda). Cryopreserved sperm or the pre-embryo of the ex-husband should not be used in the event of a divorce either, since divorce equally makes the union legal and invalid.

Another pressing problem associated with cryopreservation is the fate of frozen fertilized oocytes, if they are not used or are not needed by the owners. In Islam, human life begins in the soul, which is 120 days after conception [59].

Cryopreservation of gametes or reproductive tissue is allowed in Muslim countries before radiotherapy or chemotherapy. It can be used for conception later by their owner. Cryopreserved ovarian or testicular tissues can be re-implanted after the end of chemotherapy or radiation therapy at the request of the gonad owner. Despite over 140 babies have been born in the world after cryopreserved ovarian tissue transplantation, this promising strategy needs to be improved.
In recent decades, ART technologies including cryotechnologies have been less developed in the African continent than in other parts of the world. To date, there are no specific national laws or guidelines / regulations from national medical associations in Mali or Uganda that control the practice of assisted reproductive technology.

In 2006, the Mali National Ethics Committee on Health and Life Sciences, consisting of representatives of government, civil society and various Muslim and Christian branches, began a discussion on assisted reproductive technologies. The committee recommended a bill to the government in 2008, promoting the position of international Sunni Islam allowing the use of assisted reproductive technologies using only gametes from spouses. In 2011, the Malian government presented a modernized version, including a liberal section on life sciences, allowing the use of donor oocytes only. According to Muslim rules, the child’s origin is determined by the father [58], which makes the use of donor oocytes possible.

So oocytes and embryos are cryopreserved in Mali. Moreover, it is believed that embryos should not be destroyed, since they can all become children. They are “precious germs” not only because many resources have been invested for their production, but also because they are “precious” in the Pentecostal sense and are considered as living beings.

Among the countries of the post-Soviet period, Russia, Ukraine and Bulgaria occupy leading positions in the development of gametes and embryos cryopreservation.

Russia has the largest cryobanks. One of them is
Reprobank – high-tech cryobank of reproductive cells and tissues, including: a modern laboratory, a donor bank of sperm and oocytes, a personal bank for storing sperm, embryos and eggs [60].

The number of institutions using ART programs in Ukraine today amounts to 6 state clinics and about 45 private clinics, each of them conducts cryopreservation of reproductive cells and embryos of patients and some of them have their own donor gamete bank [61, 62]. This sphere of activity is regulated by the Order of the Ministry of Health of Ukraine. The first “in vitro” baby in Ukraine was born after transfer of fresh embryos and those cryopreserved in the IPC&C of the NAS of Ukraine in Kharkiv [63, 64]. Embryonic, placental and fetal cells and tissues are stored in the Low Temperature Bank of this institute [28, 65].

According to the Law of the Republic of Belarus "On Assisted Reproductive Technologies" of 2012, the indication for ART is the diagnosis of "infertility" and ART is performed for married couples [66]. The maximum shelf-life of donor germ cells cannot exceed 10 years. There are about 8 reproductive clinics in Belarus, but there is still no sperm bank – there are too few donors that match all criteria. Therefore, domestic medical facilities use foreign material – for example, the Embryo clinic uses Russian sperm banks for stocks.

The development of cryotechnologies in the country also affects the availability of scientific publications on this topic. And in spite of religious beliefs and a low level of economic development, in some countries there are papers about the conducted
3.5 CRYOPRESERVATION OF CORD BLOOD

Umbilical cord blood (CB) biobanking is becoming increasingly important for life quality improvement worldwide. Being a non-invasive source of hematopoietic stem cells, CB gives hope for life and recovery to children and adults with various malignant and non-malignant diseases [67]. Cord blood serum due to its components [growth factors, fibronectin, serum antiprotease (α2-macroglobulin), vitamin A, neurotrophic factors, prealbumin, oil, antioxidants] is essential for regenerative medicine [68]. Among major clinical trials using CB registered on www.clinicaltrials.gov are hematological malignancies and malignant solid tumors, Alzheimer disease, autism, traumatic brain injury, diabetes, stroke, heart and liver failure, musculoskeletal and skin diseases, metabolic disorders, etc. In this context, cord blood banks may offer not only a prolonged storage of such a valuable curing material, but also provide more human leukocyte antigen (HLA) diversity and higher proportion of different geographic origin individuals that are candidates for allogeneic transplantation [69]. Moreover, CB analysis is a promising tool in both personalized medicine, predicting various pathologies [70, 71] and population studies, assessing influence of environmental factors on the health of the population [72-75]. In these terms, hospital integrated and population biobanks play a decisive role for shaping proper diagnostics and diseases prevention programs.
Up to date around 0.7% of the total number (141594) of publications on cord blood in PubMed refers to lower-middle- and low-income countries. The interest toward cord blood and its biobanking in Africa and Asia is mainly conditioned by the urgent need for screening and healthcare programs for sickle cell disease (SCD), a severe hereditary hemoglobin disorder [76-78], malaria [79, 80] and HIV [81, 82]. In Ukraine the development of both, technologies associated with the transplantation and biobanking of cord blood hematopoietic stem cells, was predetermined by the increased cancer incidence after the disaster at the Chernobyl nuclear station in 1986. Interestingly, the first cord blood bank in Ukraine was established much earlier in 1984 at the Kharkiv city blood transfusion station in close cooperation with the Institute for Problems of Cryobiology and Cryomedicine of the National Academy of Sciences of Ukraine. It is considered to be the first European CB bank which was designed for hemotransplantation [83].

Because of the low standards of living and undeveloped insurance culture of the population, the umbilical cord blood is preserved only in 0.1–0.2% deliveries, mostly in the capital and largest cities [84, 85].

Despite the importance of cord blood biobanking, the number of studies regarding cord blood cryopreservation in the discussing countries as well as the number of specialized biobanks there, with the exception of several countries, is quite low (Fig. 3.5.1).

The main difference of CB banks in developing countries including Ukraine, from developed ones is that most of them are private (family autologous). The main reason is,
most probably, insufficient state financial support and lacking possibility of building
public biobanks. Currently there are 4 CB biobanks in Ukraine. One of them— Low
Temperature Bank of ISC of Cryobiology and Cryomedicine – was mentioned above [28].
This bank is not private.

The standard procedure, used for cord blood units preservation in biobanks,
usually includes controlled-rate slow freezing (1–2.5°C per minute) and storage at
−150°C or colder, according to the “International standards for cord blood collection,
banking, and release for administration accreditation”. Several studies in the area of
cord blood stem cells cryotechnologies were published by the scientists in India, Kenya
and Egypt. They comprise development of new cryopreservation protocols with
implementation of rapid cooling rates, vitrification and new cryoprotectant substances
[86-88] as well as assessment of samples quality after biobanking [89, 90]. In Ukraine
the number of publications and patents of this kind is quite high mainly due to the long-
time history of cryobiological researches at IPC&C. Thus development of new
methodologies for the improved cryopreservation of cord blood derived cells [91-94],
cord blood serum [95], as well as the whole cord blood [96]. Moreover applicability of
cryopreserved cord blood derived components was studied in brain aging [97], atopic
dermatitis [98], neuro-immune disorders [99] and toxic hepatitis [100] and in
regenerative medicine [101]. All these developments may allow improved
cryopreservation of cord blood and its component and could be implemented into
biobanking practice.
3.6 MEDICAL TISSUE BANKING WITHIN APPLIED CRYOBIOLOGY

The ability to use cryogenic preservation in various forms to allow storage of human tissues and organs for therapy is a clearly important application of cryobiology. However, beyond the underpinning science, the abilities to provide the necessary sustainable infrastructure, governance and training of specialized staff is an economic challenge in many countries.

It is a fact that donation of human tissues and organs increases significantly when tissue banks and organ transplant organizations, established within the country, work together in the procurement of human organs and tissues. Transplantation can improve the quality of life and reduce the total cost of care for patients. Nevertheless, in LMICs these procedures are still not accessible to the entire population. These countries might benefit from the experience of other LMICs that have increased access through successful transplantation programs, from guidance on needs assessment, and from regional and global partnerships in developing transplant programs. A tissue banking organization is a very complex system that needs high technical expertise and skilled personnel for proper functioning in a dedicated facility. A small lapse/deviation from the established protocol may lead to precious tissues loss and/or harm to recipients, as well as the risk of transmission of deadly diseases and tumors. Worldwide there are hundreds of tissue banks and organ transplant organizations operating in different countries, some of them, with considerable experience in the use of sterilized tissues in specific medical treatments and the field of organ transplantation [125].
Strict tissue transplant acts and stringent regulations help to streamline the whole process of tissue banking safe for recipients and to community as whole. Each country has its own standard operating procedure, guidelines and regulations to control the selection of donors, microbiological and serological tests, viability of tissues and ethical considerations. In 2019, the 164th Session of the Executive Committee of WHO and PAHO published a guideline for an equitable access to organ, tissue and cell transplants [126]. The Guiding Principles are intended to provide an orderly, ethical and acceptable framework for the procurement and transplantation of human cells, tissues and organs for therapeutic purposes. Despite the fact that international collaboration is an essential tool for scientific development of LMICs, clear ethical and legal regulations are central to avoid exploitation in research involving vulnerable populations [124].

According to the Global Observatory on Donation and Transplantation (http://www.transplant-observatory.org/), between 2016 and 2018, the Americas and Europe are the leading WHO regions regarding transplant per million of population (58.6 and 56.6, respectively). These rates are far away from those found in LMICs, as an example, Honduras 1.4, Nicaragua 2.5 (within America); Sudan and Algeria 6.6, Ethiopia and Kenya 0.4, and Nigeria 0.7 (Africa). Lack of statistics is also associated with absence of tissue banking and organ transplant organization in other LMICs. To overcome this, the promotion of the broadest possible cooperation between tissue banks, organ transplant organizations, hospitals, and other donors sources in the country; and the establishment of a National Tissue and Organ Transplant Office with the harmonization of laws and regulations in the field of transplantation, among others, are concrete
proposals that deserves the attention of the national competent health authorities of
the different countries [125]. The following discussion outlines how this has been
approached in Latin America, with Argentina as an exemplar.

**Tissue Banking in Argentina**

The first attempts to establish tissue banks in Argentina were promoted by
personal initiative of specialist formed mainly in Europe and the United States. Initial
success in corneal and bone tissue transplant between late 1920s and 1950s
encouraged the creation of tissue banks located mainly on public hospitals (such as the
National Corneal and Vessels Bank). Such institutions worked mainly as closed
compartments during the 1960s and 1970s. Research and surgical procedures were not
regulated and kept being results of professional’s personal initiatives. This unregulated
approach facilitated the direct application of research into clinical practice mainly due to
spatial and temporal coincidence of research and medical teams. An actual example
(and not the only one) of such coincidence is the Pediatric Hospital “J.P. Garrahan” in
Buenos Aires. In this institution, three main actors of the tissue banking specialty
coexist: the bank itself, a research group and the medical groups for ablation and
implantation.

In 1977 the “Unique Center of Ablation and Implantation”, later named “Unique
Central Institute for Ablation and Implantation” (CUCAI/INCUCAl, website:
www.incucai.gov.ar) was created by law as an intent of regulate the activity. Since then,
public and private initiatives in tissue banking are subjected to strict standardization of
procedures. Any improvement, modification or revision of accepted protocols must be
approved by this national funded regulatory authority. A further decentralization of regulatory competence was achieved by the creation of regional or provincial representations of INCUCAI. Nevertheless, there is no regulation at regional or provincial level which contradicts the ones announced by the national level [102]. In theory, technical competence of INCUCAI should help to improve translation of basic and applied research to the clinical applications by constituting the only legal path of such translation.

Although decentralization improved the administration, regulation, and guaranteed the availability of competent medical teams in every major public health institute of the country, tissue banking remained concentrated in the three main urban conglomerates of the country. Local regulation also promoted the creation of a number of private tissue banks which usually function within private health clinics with limited habilitation from the regional authority at best. Information and statistics about procedures of such banks are scarce and usually not in public domain. Public institutions represent 50% (17 of 34) of legally habilitated tissue banks in Argentina and are located within public hospitals infrastructure [103]. Figure 3.6.1 shows public/private distribution of legally habilitated tissue banks in Argentina grouped by tissue type.

Nowadays more than 220 tissue banking facilities are present among Latin American countries. In general, tissue banks are regulated and controlled by a specific structure within the frame of the National System of Health, according to the National Law of Donation and Transplantation of organ, tissue and cells of each country.
Therefore regulatory systems, training programs and other issues related to
donation and transplantation varies among countries. With the purpose of reducing
these differences, several associations and networks were created, being Argentina an
important member of all of them. Between them the Latin American Association of
Tissue Banks (ALABAT), the Red Consejo Iberoamericano de Donación y Transplantes
(RCIDT, Ibero American Network Council of Donation and Transplant) and DONASUR can
be named.

The RCIDT composed by 19 countries of Latin America plus Spain and Portugal
has full support and technical collaboration from the Pan American Health Organization
(PAHO/WHO). DONASUR is the official record of donation and transplantation of the
countries that constitute the MERCOSUR, the officially Southern Common Market. This
record was created by an agreement of the meeting of Ministers of Health of
MERCOSUR, and implemented in a multi-language web platform (www.donasur.net).
According to RCIDT, Argentina and Brazil are the only Latin-American countries in which
the existent regulation also includes technical guidelines [104] for procedures, including
storage and transportation.

Adequate supply of Liquid Nitrogen is fundamental when running a tissue bank.
Global companies (Linde Group and Air Liquid, in example) have representatives in
South America, and they provide medical gases and cryogenics to in major cities. For
supply in small cities or specifics applications, like small scale tissue banks, plant seed
banks, sperm banks, etc., there are private providers. Although these are reliable in
terms of provision, there is a lack of security in terms of transportation. As an example,
they are using particular cars for transportation (Guibert, E. personal observation). In recent years a number of research institutes are producing liquid nitrogen in their own installations covering the cost for not only for equipment installation but also for capacitating of the involved personal.

In general, applied cryopreservation techniques in tissue banks vary from controlled freezing to −80°C and liquid nitrogen vapor storage (at −170°C) in the case of heart valves, or storage at −80°C mechanical freezers of amniotic membrane [105]. All the procedures, disregarding the applied methodology, are divided in two stages. Tissues are initially processed according to necessities and remain in quarantine until microbiological safety is assessed and all legal requirements are accomplished. In some cases, cryopreserved material is sterilized using gamma radiation. This procedure requires proper transportation of cryopreserved tissues to specialized facilities and back to banking facilities. Once all safety and legal conditions are met, tissues are considered suitable to implant and placed on final storage conditions until needed.

Related to the equipment, in general, tissue banks are correctly equipped, with an adequate maintenance of the equipment. However, due to financial problems, associated to the annual inflation rate, it is very hard to renovate them. To handle this equipment, the presence of skilled personnel is fundamental and here is, perhaps, the biggest deficit: the lack of training or teachers to provide it. In the field of research, practical solutions always emerge that replace or cover the operation of very onerous equipment. As an example, the thermostated chamber for mounting on a microscope
stage and allows observing specimens at low temperatures [106] or the controlled cooling system [107].

During the last thirty years governments and professionals from Latin America region, including Argentina, have made great efforts to implement and develop high quality tissue banks. In order to achieve this goal, different training courses on Tissue Banking and Radiation for bank operators and medical doctors were carried out by the International Atomic Energy Agency (IAEA), the National Atomic Energy Commission (CNEA) and the Faculty of Medicine of Buenos Aires, with the collaboration of INCUCAI [108]. In the year 1999 Argentina was the IAEA’s Training Centre for Latin America.

To address this point, in Argentina, the laboratory associated to the UNESCO Chair, the Argentina – Italia Binational Center for Applied and Clinical Cryobiology Research (CAIC), has offered, as a path for academic translation: a specialization in biopreservation, forming human resources capable of directing tissue banks. The objective is to form people who will be in charge or will have the possibility to maintain a biobank (including although not exclusively, tissue banks), providing a holistic understanding of fundamental cryobiology and its application, in order to satisfy current mentioned needs of health and industrial actors. The actual syllabus and dedication time of the specialization course is resented in table 3.6.1.

3.7 NEW APPROACHES IN CRYOBIOLGY

The ability to use cryogenic preservation in various forms to allow storage of human tissues and organs for therapy is a clearly important application of cryobiology.
However, beyond the underpinning science, the abilities to provide the necessary sustainable infrastructure, governance and training of specialized staff is an economic challenge in many countries.

As global recognition of the importance of effective cryopreservation to both medicine and fundamental biological research increases, a new generation of cryobiological techniques are emerging. Many of these methods are fundamentally re-assessing the approach to cryopreservation innovated by Polge, Pegg, etc., which has traditionally relied on high doses of cytotoxic cryoprotectant agents (CPAs) and storage in cryogens at deep temperatures (−80°C and below). We will herein discuss select new approaches that, whilst advancing the state of the field in a purely scientific sense, are also low-cost or low-complexity and hold significant potential translatability to countries with middle- and low-income economies. For a more technical discussion of emergent cryopreservation techniques across the board, the reader is directed towards a recent review by Taylor et al [110]

High Subzero Techniques

Traditionally cryopreservation protocols have targeted the deep cryogen temperature regimes (−196°C to −80°C) at which cellular metabolism is near-totally arrested and years-long preservation is enabled. While this regime remains an ultimate target for the cryobiology community, it has recently been acknowledged that even short-term (on the scale of days or weeks) preservation of sensitive biologics such as complex tissues or organs could be transformational, enabling intercontinental transportation of these materials, unprecedented sharing of biological resources for
both medicine and research between countries, etc. [111]. Thus the development of effective preservation techniques that operate in the high-sub-zero Celsius temperature range (−20°C to −3°C) could both reduce the economic burden of preservation by eliminating the materials and handling costs of cryogens such as LN2 and enhance the ability of countries with high-income economies and state-of-the-art cryopreservation infrastructure to redirect biological resources to less well-equipped countries across the globe. Notable emergent high subzero methods include:

**Supercooling**

When an aqueous solution remains liquid at temperatures below its equilibrium freezing point, it exists in a metastable “supercooled” state. In this state the thermodynamic driving forces that propel phase change are insufficiently strong to cause immediate spontaneous freezing, and a delicate pseudo-equilibrium is achieved. The capacity for supercooling of a given solution is a function of a wide-range of parameters, including volume, temperature, surface interactions with its container or surroundings, solutes, etc., and our current ability to rigorously predict supercooling efficacy in solutions containing various solutes and biological materials is limited. However, early experimental work applying supercooling to the preservation of various biologics has shown tremendous potential.

Huang et al. demonstrated a simple first-order procedure in which they capped vials of cellular suspensions with an oil phase (commercially available mineral and vegetable oils) in order to eliminate the air-water interface (which was determined to provide a prominent nucleation site for ice) and achieved supercooling of clinically
relevant volumes (up to 100 ml) for up to 100 days at temperatures as deep as −20°C, reporting human red blood cell viabilities above 90% [112]. Applying supercooling in combination with machine perfusion and light concentrations of non-toxic cryoprotectants 3-OMG and PEG, Berensden et al. developed a protocol enabling 4-day preservation of rat livers at −6°C, representing a tripling of the current clinically achievable preservation period [113]. While the net success of this preservation protocol cannot be extricated from the technologically-complex machine perfusion step, it is regardless a strong indicator of the potential of supercooling in preservation whole-organ or complex tissue applications. Amir et al. further achieved a 5-fold increase in preservability for rat hearts using anti-freeze proteins derived from Arctic fish, which enable a unique state of stable supercooling at −1.3°C [114, 115]. This suite of varying supercooling protocols represents a “low-tech” approach to cryopreservation, which requires little-to-no specialized equipment and may be achieved to varying degrees in any aqueous solution. While supercooling is a non-equilibrium technique (e.g. avoidance of freezing is likelihood as opposed to an assurance), rigorous effort to document the capacity for supercooling across the wide gamut of oft-employed preservation solutions could provide a roadmap enabling the development of high-efficacy, low-cost supercooling protocols.

**Isochoric Preservation**

Life on planet Earth has evolved as a constant-pressure (isobaric) thermodynamic system, and thus the overwhelming majority of cryobiology research has been conducted at constant (typically atmospheric) pressure. Rubinsky et al.
recently considered the thermodynamic implications of instead conducting the freezing process under constant volume conditions, or in an isochoric system, and identified tremendous differences in the equilibrium behaviors of water and ice in the high-subzero temperature range [116-118]. Under isochoric conditions (e.g. in a system of constrained volume with no highly compressible components such as air), ice and water will form a controlled two-phase equilibrium as a result of the enhanced hydrostatic pressure produced by expansion during freezing, which enables stable, ice-free preservation of biologics in the remaining liquid phase. This preservation is realized experimentally in low-cost, low-complexity “isochoric chambers” comprised of simple stainless-steel or titanium chambers equipped with elementary pressure monitoring, and represents a stable-equilibrium alternative to supercooling in the high-subzero regime.

Preliminary results in the isochoric preservation of biological matter have emerged only recently, but show promise and applicability across a range of systems. Wan et al., found that isochoric preservation of rat hearts at −4°C improved preservation quality compared to standard hypothermic storage in an ice bath, displaying reduced interstitial edema and vascular injury, and Powell-Palm et al. preserved rat pancreatic islets for up to 3 days with high viability at −3°C [119, 120]. Additional works have also demonstrated the efficacy of isochoric preservation using a range of biological systems with non-medical applications, including fish muscle, sweet cherry, and the model organism *Caenorhabditis elegans* [121-123]. Significantly, all of these protocols were undertaken without the requirement of any form of chemical
cryoprotectant, removing both the material cost and the requirement of potentially-complex CPA-introduction and removal steps, and further indicating their potential for use in resource-limited environments. It is also important to note that isochoric preservation is an equilibrium technique, and thus does not face thermodynamic limitations in scaling or stability. Principal challenges involve further mapping the fundamental thermodynamic behaviours of the systems and clarifying the potentially detrimental effects of enhanced hydrostatic pressure during preservation.

All of the results discussed in this section, which represent significant improvements upon currently employed preservation standards, are achieved in the absence of cryogens, at temperatures achievable in a household freezer. This suggests that an immediate potential path forward for cryobiology research and practice in resource-limited areas may be charted by techniques that operate within the high-subzero regime.

**SUMMARY AND FUTURE PERSPECTIVES**

It is clear from our assessments of the various outlets for translational cryobiology that some knowledge about the benefits of cryotechnologies has spread in a truly global fashion. However, access to the full range of cryotechnologies may not be entirely assured in any one country. Also, some cryotechnologies may only be available within major cities or population centers, probably driven by local economic factors. Never-the-less, a few main points can be set out.
1) Even in the most economically-challenged countries, certain areas of science, production, technologies etc. can be highly developed. The level of their development isn’t always determined by the general economic activity in the country.

2) Popularization of cryobiology worldwide, including low-income countries is limited by certain factors:

- Cryobiological techniques cannot be implemented without providing people with information about importance of this science and its applications;
- Local infrastructure needs to be in place to provide suitable cryo-equipments and access to reliable cryogens;

There are two suggested broad directions for popularization:

- cryobiology for health care purposes;
- cryobiology for preservation of genetic resources, biodiversity, crop management and sustainable exploitation of the natural environment.

All these themes will require knowledge transfer between economically-privileged countries and LMICs.

3) Education in Cryobiology is necessary for

- training of specialists in cryobiological techniques;
- raising awareness within government and local political / societal organizations of the potential benefits which can accrue from correct applications of translational cryobiology;
- popularization of cryobiology.

4) UNESCO Chair in Cryobiology is able to:
– connect specialists and all interested individuals and organizations from different countries;

– act as a hub to share information and provide education in cryobiology (p. 2 and 3).

5) UNESCO Chair should cooperate with other similar organizations in other countries. (For example, the IPC&C began working at this and negotiated the collaboration with Armenian colleagues http://www.biophys.am/about-lsiepc/).

6) Scientific societies working in topic areas which involve cryotechnology, for example in reproductive medicine, are beginning to hold more regional meetings such that local expertise and knowledge can be better spread. They should also be encouraged to develop public awareness sessions about the topics, which in turn can raise local media interest in translational cryobiology.

ACKNOWLEDGMENT

A report facilitated by the UNESCO Chair in Cryobiology hosted by the Institute for Problems of Cryobiology and Cryomedicine, National Academy of Sciences of Ukraine
<table>
<thead>
<tr>
<th>Abbreviation</th>
<th>Description</th>
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<tr>
<td>ALABAT</td>
<td>Latin American Association of Tissue Banks</td>
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<td>ART</td>
<td>assisted reproductive technologies</td>
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<td>CAIC</td>
<td>Italia Binational Center for Applied and Clinical Cryobiology Research</td>
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<td>CB</td>
<td>umbilical cord blood</td>
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<td>CNEA</td>
<td>National Atomic Energy Commission</td>
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<td>CPAs</td>
<td>cryoprotectant agents</td>
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<td>CUCAI/</td>
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<td>INCUCAI</td>
<td>Unique Central Institute for Ablation and Implantation</td>
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<td>DNA</td>
<td>deoxyribonucleic acid</td>
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<tr>
<td>FAO</td>
<td>Food and Agriculture Organization</td>
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<td>HES</td>
<td>hydroxyethyl starch</td>
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<td>HLA</td>
<td>human leukocyte antigen</td>
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<td>IAEA</td>
<td>International Atomic Energy Agency</td>
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<td>IVF IPC&amp;C</td>
<td>in vitro fertilization</td>
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<td>IPC&amp;C</td>
<td>Institute for Problems of Cryobiology and Cryomedicine</td>
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<tr>
<td>ISC</td>
<td>Interdepartmental Scientific Center</td>
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<tr>
<td>LIEC</td>
<td>low-income economies country</td>
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<td>LN₂</td>
<td>liquid nitrogen</td>
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LMICs low and middle income countries
LMIEC lower-middle income economies country
PVA polyvinyl alcohol
RCIDT Red Consejo Iberoamericano de Donación y Transplantes
TTI transfusion-transmitted infection
UMIEC upper-middle income economies country
WHO World Health Organization
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Figure Captions List

Fig. 3.5.1 Number of cord blood cryotechnology publications in Pubmed and biobanks (low- and lower-middle income countries)

Fig. 3.6.1 Distribution of public and private share on habilitated tissue banks in Argentina. OT: Ocular Tissue; OAT: Osteoarticular tissue; CVT: Cardiovascular Tissue; AM: Amniotic Membrane; HPC: Hematopoietic Progenitor Cells; S: Skin. Total number of tissue banks in Argentina: 34
Table Caption List

Table 3.3.1  Number of countries with animal genetic resources preservation programs

Table 3.6.1  Syllabus for the Biopreservation Specialization in Argentina. Courses are grouped in 4 months blocks (grey and white blocks). (h): presential course hours
**Fig. 3.5.1.** Number of cord blood cryotechnology publications in Pubmed and biobanks (low- and lower-middle income countries)
**Fig. 3.6.1.** Distribution of public and private share on habilitated tissue banks in Argentina. OT: Ocular Tissue; OAT: Osteoarticular tissue; CVT: Cardiovascular Tissue; AM: Amniotic Membrane; HPC: Hematopoietic Progenitor Cells; S: Skin. Total number of tissue banks in Argentina: 34
### Table 3.3.1. Number of countries with animal genetic resources preservation programs

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<td>Southern</td>
<td>11</td>
<td>4 (36%)</td>
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<td>4 (33%)</td>
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<td>42</td>
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<td>12 (30%)</td>
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<td>Asia Central</td>
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<td>4</td>
<td>2 (50%)</td>
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<tr>
<td></td>
<td>East</td>
<td>4</td>
<td>3 (75%)</td>
<td>4</td>
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<td>6</td>
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<tr>
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<td>5 (50%)</td>
<td>8</td>
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</tr>
<tr>
<td></td>
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<td>9</td>
<td>1 (11%)</td>
<td>5</td>
<td>3 (60%)</td>
</tr>
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<td>6 (27%)</td>
<td>18</td>
<td>11 (61%)</td>
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</tr>
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<td>1</td>
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<tr>
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<td>7</td>
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<td>World</td>
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<td>55 (37%)</td>
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<td>70 (55%)</td>
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Note: Figures refer to the proportion of countries reporting preservation activities for at least one species. Source: [53].
Table 3.6.1. Syllabus for the Biopreservation Specialization in Argentina. Courses are grouped in 4 months blocks (grey and white blocks). (h): presential course hours

<table>
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<tr>
<th>Course Id Code</th>
<th>Course Name</th>
<th>Theory (h)</th>
<th>Practice (h)</th>
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