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OPINION

Long-term storage of gametes and gonadal tissues at room temperatures: the end of the ice age?

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Abstract

Long-term preservation of viable spermatozoa, eggs, embryos, and gonadal tissues of good quality is essential in human reproductive medicine and for the population management of livestock, laboratory, and wild species. Instead of using freezing temperatures, encouraging findings indicate that structures and functions of gametes or gonadal tissues can be suspended in trehalose glass after dehydration and then preserved at supra-zero temperatures. As a new era in fertility preservation and biobanking is about to start, the advantages, needs, and implications of germplasm storage at room temperatures must be carefully examined. Although very promising, the development of alternate biobanking strategies does not necessarily mean that the end of the "ice age" (cryopreservation) is near.

Keywords Cryopreservation · Freezing temperatures · Biobanking · Anhydrobiosis · Room temperature storage

The need for germplasm biobanking is increasing, but current cryo-storage strategies are often constraining and not always sustainable

Preserving biomaterials of good quality for the long-term is essential in multiple scientific disciplines. There is a specific interest in preserving spermatozoa, eggs, embryos, and gonadal tissues (so-called germplasms) in human reproductive medicine, livestock production, laboratory animal management, and wild species conservation [1]. Demand for safe germplasm storage in combination with assisted reproductive technologies (ART) is also steadily increasing in those different areas [2, 3].

Cellular as well as molecular structures and functions of samples are currently stabilized (suspended animation) by freezing temperatures which ensure long-term longevity and quality of the biomaterials [4]. Spermatozoa, oocytes, and embryos from common species (human included) can survive the various stresses associated with conventional cryopreservation, which involves exposure to toxic cryoprotectant(s), cooling/freezing, liquid nitrogen storage (at − 196 °C), and thawing/rewarming. However, there is an extreme sensitivity and specificities of tissues, cells, organelles, and DNA to cryoprotectants and low-temperature exposures, lessons we have learned from years of studies in different model species [5, 6]. In terms of innovation in long-term preservation, vitrification was reported > 35 years ago to overcome the issues related to ice crystal formation in mouse embryos [7]. Since then, very little attention has been directed to novel means for storing any type of germplasm for the long-term.

Importantly, electrical subzero freezers or liquid nitrogen containers require complex maintenance, alarm systems, and specialized rooms with back-up power and controlled environment to mitigate the heat produced by the freezer compressors or to evacuate nitrogen vapors. Unfortunately, facilities with sustained electrical power and liquid nitrogen are expensive and not always affordable or readily available in some regions of the world. Additionally, cryogenic temperatures are hard on thermo-sensors that are used to monitor liquid nitrogen storage. Storage containers also are not at always properly equipped, so visual inspection is necessary

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to check the levels of liquid nitrogen, which is highly constraining and not sustainable. Thus, cryo-storage systems are prone to failures — from equipment breakdown to human error — which, recently, has led to dramatic sample losses in human fertility clinics and research laboratories.

The search for alternate solutions to cryo-storage has started several years ago and has led to recent breakthroughs

In nature, certain nematodes, tardigrades, insects, and brine shrimp survive extreme cellular water loss via a natural process called "anhydrobiosis" — a term first used by Alfred Giard in 1894 [8, 9]. Cellular and molecular activities are suspended above freezing temperatures instead of occurring at subzero temperatures. In addition to slowing down metabolism and producing critical components, one of the keys to reach and survive dry conditions relies on the organism capacity to synthesize and accumulate intracellular disaccharides (mainly trehalose) while losing water content. Biostabilization is then primarily reached by the formation of a trehalose glass — a high viscosity liquid that immobilizes enzymes and prevents chemical activities — at supra-zero temperatures. Major advantages of natural sugars like trehalose are their low toxicity and high glass transition temperature compared to conventional cryoprotectants such as dimethyl sulfoxide, ethylene glycol, or 1,2-propanediol [10].

Desiccation is very common in seeds or pollen and is currently better understood than in mammalian sperm cells and eggs. Lessons learned from anhydrobiotic organisms provided several candidate genes [11], whose role in desiccation tolerance is being assessed by several laboratories [12–14]. Unfortunately, desiccation genes or relative analogs are not present in vertebrate genomes; therefore, directly supplying "xero-protectants" to the cells is mandatory before dehydration, given that water deprivation remains a fatal threat. Importantly, other protective molecules, such as the late embryogenesis abundant proteins, also are found in plants and could confer water stress tolerance to mammalian somatic cells [14].

At present, sample drying is mainly achieved by freeze-drying, which is the sublimation of the ice after freezing (under low pressure) and then removal of residual water by desorption. Several studies have confirmed the suitability of freeze-dry for mammalian sperm preservation, for instance, in the mouse [15, 16], ram [17, 18], stallion [19], or bull [20] (embryo production or live births following ICSI). Lyophilization or freeze-drying of sperm cells in trehalose has also been successful in human [21, 22]. Recently, partial freeze/dried human spermatozoa was successfully rehydrated and utilized for ICSI, leading to the production of normal euploid human blastocysts [23]. However, besides sperm cells, only one report exists about lyophilization of germinal vesicles in the porcine model [24]. A recent report on sheep ovarian tissue lyophilization also is promising but shows the vast amount of work that is needed to preserve fully functional gonadal tissues [25].

Over the past decade, microwave-assisted desiccation (without preliminary freezing) in the presence of trehalose has been explored using germplasms from domestic cats [26, 27]. Germinal vesicles, sperm, ovarian tissues, and testicular tissues [26, 28–31] have been successfully preserved and stored at non-cryogenic temperatures in sealed pockets. A simple rehydration is then sufficient to reanimate and use the samples.

Overall, these encouraging results clearly show that structures and, more importantly, functions of gametes and gonadal tissues can be suspended in trehalose glass and potentially be preserved for the long-term at supra-zero temperatures. In the meantime, we also learned that there is still a need for environmental control during storage (in terms of temperature and humidity levels).

Despite encouraging advances in storage at room temperatures, more research in specific directions is needed

So far, the technical evidence of a better dehydration method is missing. A recent survey demonstrated that, despite 10 major dehydration techniques are in theory available, lyophilization remains the preferred method; however, only few data exist on the other desiccation strategies [1]. Although we are inspired by natural "anhydrobiosis," none of the organisms mentioned above undergoes freezing followed by low-pressure sublimation of ice.

Moreover, lyophilization is performed using devices directly inspired from the pharmaceutical or food processing industry, where large volumes of materials are dried (which might lead to additional mechanical stresses to microscopic cells and small tissue biopsies). There is an urgent need to optimize the dehydration process and storage containers with devices adapted to the size of each sample. Thus, it is far from proven that lyophilization is the golden standard procedure for water removal in mammalian germplasms. Likewise, rehydration remains largely unstudied and is as important as the drying process.

Therefore, joint efforts between bioengineers and cryobiologists are required for the development of dryers that better fit the needs of mammalian cells and gametes. More choices in small size and adapted bench-top dryers would be convenient to advance faster our knowledge on reversible drying of germplasms. More research in experimental animal models also is warranted before translating new knowledge to other species, including humans.

The field is advancing rapidly as shown by the increasing success of embryonic development following injection of dried/rehydrated spermatozoa. Even minor technical improvements affect the efficiency dramatically [32], and if we consider that ICSI (the compulsory technique used to fertilize oocytes with the motionless dry spermatozoa) performs poorly in large animal models [18], it is likely that better outcomes could be expected in humans since sperm injection is a nowadays a routine technique IVF clinics. While there might be a growing interest by human ART experts in adopting dry storage for human spermatozoa [22], many more data are required on the production of live offspring with freeze-dried spermatozoa, including their health later in life, even at the epigenetic/genetic level to exclude long-term side effects on DNA caused by desiccation and storage at room temperatures.

Room temperature storage will profoundly change biobanking logistics and operations

Regardless of the implications of drying techniques to sample processes, maintenance, and curation, we will still have to ask the same essential questions to any new emerging banking effort: what are we storing? Why are we storing it? What are we storing in it? How many do we want to store? For how long?

Of course, there are enormous management advantages of desiccating and storing germplasms at ambient temperatures. First, it will considerably reduce processing and storage costs by simplifying the preservation methods, decreasing specialized space/infrastructure needs, and avoiding liquid nitrogen purchase. Second, the biosecurity (prevention of pathogen transmission) of storage at supra-zero temperatures in individual containers should be higher than samples within the same liquid nitrogen vat. Third, it will facilitate transport of biomaterials between locations and even offer patients the option of at-home storage for their own samples. Lastly, while moisture content will have to be maintained to a low level to prevent degradation (in vacuum sealed pockets, for example), samples will be more resilient to variations of temperatures during storage than frozen samples.

Even though there will be less constraints in terms of the location, new storage facilities at room temperatures will still require environmental control, sample accessioning, and safety. We will also need new sample holders and identification/labelling methodologies for dried samples. In sum, a different set of standard operating procedures will have to be developed.

Room temperature storage will also help to develop the concept of de-centralized biobanks (or home storage) that involves less liability than centralized biobanks. Samples would be closer to the end-users and could be easily stored for short duration. However, new sets of ethical aspects and issues of proper use (risk of parallel markets) may have to be anticipated.

Is it the end of the ice age?

As ARTs are constantly evolving, it is expected that alternate methods of germplasm preservation will be more prevalent in the coming decades either in biomedical science, livestock industry, biodiversity conservation, or human reproductive medicine. Starting with sperm storage at room temperatures, new options will be progressively integrated into routine practice and have a significant impact on biobanking operations. It will obviously take more time for room storage of oocytes and gonadal tissues to be developed and implemented.

While there are obvious advantages of storing germplasms at ambient temperatures, we expect that cryo-banks will remain the primary biobanking strategy for a while (especially for current frozen samples stored that will have to be maintained), even if drying techniques are fully developed for all types of germplasms. While we may transition to more biobanks at room temperatures, there might still be advantages of maintaining a mix of cryo- and xero-banks rather than switching to a single storage option.

Declarations

Conflict of interest The authors declare no competing interests.

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