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# Highly efficient vitrification for cryopreservation of human oocytes and embryos: The Cryotop method

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**Abstract**

Vitrification is frequently referred to as a novel technology of cryopreservation in embryology, although some young embryologists were born after its first successful application. Unfortunately, in spite of the accumulated evidence regarding its enormous potential value, most domestic animal and human laboratories use exclusively the traditional slow-rate freezing with its compromised efficiency and inconsistency. The purpose of this paper is to clarify terms and conditions, to summarize arguments supporting or disapproving the use of vitrification, and to outline its role among assisted reproductive technologies. To provide evidence for the potential significance of vitrification, achievements with the Cryotop technology, an advanced version of the ''minimal volume approaches'' is analyzed. This technology alone has resulted in more healthy babies after cryopreservation of blastocysts than any other vitrification technique, and more successful human oocyte vitrification resulting in normal births than any other cryopreservation method. The value of this method is also demonstrated by achievements in the field of domestic animal embryology. A modification of the technique using a hermetically sealed container for storage may help to eliminate potential dangers of disease transmission and open the way for widespread application for cryopreservation at all phases of oocyte and preimplantation embryo development in mammals.


*Keywords:* Cryodevice; Blastocyst; Pregnancy

## 1. Introduction

One of the eternal ambitions of humankind is to overcome limits created by dimensions. After initial signs of successes to overcome horizontal barriers with railways almost 200 years ago, a real breakthrough only occurred in the 20th century, accompanied by expansion also into the third dimension, i.e. into the air and also into space. Today, the once demanding 42 km horizontal distance presents a challenge only for a marathon runner; practically all points of the globe can be reached in less than 48 h, while traveling at most at 10 km above the surface of the earth. In parallel, the astonishing advancement of information technology has helped us to eliminate virtually all remaining distances by enabling communication between continents exactly as easily as between neighboring offices.

Compared to that, almost nothing has happened with the fourth, maybe even more important dimension: time. We cannot travel forwards nor backwards, cannot slow down our limited available time, and cannot probably even use it better than previous generations. Among the very limited achievements in this debate we may list the cooling and later deep-freezing of biological materials, mainly food to slow down post mortem degradation; and hibernation to expand the life-span of living tissues and organs under temporarily unfavorable conditions.

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However, the second half of the last century has resulted in considerable advances allowing the biological clock of live objects to be stopped for an unlimited (or practically unlimited) period, made possible by cooling them to extremely low temperatures, below −150 °C. This approach was first applied to simple structures including bacteria or single cells, and then advanced to some multicellular organisms and organs. Currently, major limitations are size and complexity: generally the smaller the sample and the simpler its structure, the better the chances for successful cryopreservation. Accordingly, embryology is in a rather privileged situation because of the relatively small size of the biological samples we work with, although even seemingly negligible variations in size may cause considerable differences in survival rates.

Since the middle of the last century, in parallel with the development of in vitro reproductive technologies, remarkable successes have been achieved in cryopreservation of spermatozoa, embryos and oocytes, enabling us to stop and restart time both shortly before and after fertilization. Apart from the biological success, this possibility may have profound philosophic and moral consequences, especially when human material is concerned. Among other manipulations, stopping of time seem to be tolerable in the case of gametes, but it has been forbidden for embryos in many countries for ethical and legal reasons. As a curious consequence, to avoid restrictions with serious consequences, developmental stages have been re-defined in some of these countries by replacing the name ‘zygotes’ or ‘one-cell embryos’ with the term ‘pronuclear stage oocytes’, an expression that is rather paradoxical for experimental and domestic animal embryologists, but one that permits more legal freedom in assisted reproduction in humans. Another example is the euphemistic category of ‘‘pre-embryo’’ in human assisted reproduction.

In spite of these controversial situations, the need for efficient cryopreservation of oocytes and embryos is enormous both for theoretical and practical reasons. However, in spite of the vast efforts invested, advances are rather slow. The main problems are the lack of consistency [1] as well as differences in survival and developmental rates after warming between species, developmental stages and quality. While the latter is easy to understand, we have only partial and not entirely supported information regarding the reasons for the lack of consistency in results. On the other hand, basic research in this field follows only empirically obtained advances: instead of indicating future directions for improvements, its main role is restricted to explaining retrospectively the achievements (a very recent example for this situation is the history of the discovery of somatic cell nuclear transfer.

In contrast to spermatozoa, where slow-rate freezing is almost exclusively used for cryopreservation and alternative methods are applied only at an experimental level [2], in cryopreservation of mammalian embryos and especially oocytes, vitrification has become a viable and promising alternative to traditional approaches. The convincing evidence that has been accumulated regarding the huge potential importance of vitrification has been reviewed recently [3–6]. Consequently, this paper will focus only on the latest achievements, and will discuss in detail the Cryotop vitrification method that has resulted in the highest number of babies born after vitrification of human embryos and after cryopreservation of human oocytes worldwide, and is now also successfully applied in various areas of animal biotechnology.

## 2. Vitrification

In spite of the fact that mammalian embryo and oocyte vitrification was the subject of more than 500 publications in the past 10 years, and no comparison in any systems has proved its efficiency to be inferior to that of traditional freezing, vitrification is still regarded as experimental [3,4] and its practical use is restricted to a very few human and domestic animal embryology laboratories. Apart from the theoretical and practical problems, many misunderstandings also hamper its large-scale application. There are problems even with the definition. Vitrification is just a vitreous, transparent, ice-free solidification of water-based solutions at subzero temperatures [7]. Accordingly, even the first paper describing successful cryopreservation of mammalian spermatozoa used this term in a context that is not applicable today [4,8]. In some papers, vitrification is described as the result of extremely high cryoprotectant concentrations and extremely high cooling rates. However, vitrification does not necessarily require high cryoprotectant concentrations, because even pure water can be vitrified if the cooling rate is high enough ($-10^7$ °C/s) [9] and, on the other hand, with concentrated cryoprotectant solutions, vitrification can also be achieved with a moderate or even slow cooling rate [10]. Other papers regard direct contact between liquid nitrogen and the embryo-oocyte containing solution as a prerequisite of vitrification, although it is just one possibility for increasing cooling rates that may permit decreasing the concentration of cryoprotectants and minimizing their potential toxic and osmotic effects. Many vitrification techniques (for example those earlier forms based on sealed 0.25 ml insemination straws or