

VITRIFICATION IN HUMAN A.R.T.: LESSONS FROM THE LABORATORY

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Successful cryopreservation of gametes in animal reproduction has a track record of over more than 60 years. By using the vast amount of knowledge on cryopreservation collected over the years, led to the first pregnancy in humans derived from frozen-thawed embryos in 1983/84. Since then the impact of cryopreservation on the growth and improved efficiency of assisted reproduction in humans became increasingly appreciated. With nearly 700,000 babies born worldwide after cryopreservation, this techniques has become well established as a widely used routine procedure. The aim for cryopreserving cells successfully at -196°C can be summarized as follows: (1) arresting the metabolism, while maintaining the structural and genetic integrity of the cell; (2) to achieve acceptable survival rate post thaw; (3) the protocol/technique must be reliable and repeatable. Furthermore, all cryopreservation methods and protocols are developed such that ice crystals will not appear and grow inside the cells or tissues of the specimen being cryopreserved. One way to achieve an ice-crystal free state is to establish a glassy or vitreous state, called vitrification. In order to decrease the likelihood of lethal ice crystals forming through the crystalline phase by inducing the creation of amorphous intracellular parts, three characteristics have to be considered as important: (1) minimal sample volume (submicroliters); (2) high cooling and warming rates (more than 2,500°C/min), and (3) a cooling time of less than 2 seconds. Although initially reported in 1985 as a successful cryopreservation approach for mouse embryos, vitrification has taken a backseat in human assisted reproduction. However, the practical advantages of this cryopreservation method quickly have caught the attention of many ART laboratories as a feasible alternative to traditional slow freezing methods. Since 1985, 1,500 publications can be found on the topic of “vitrification”, which is further evidence of the spark in interest in this cryopreservation procedure. One “drawback” considered by embryologists who are not familiar with vitrification technique, is higher concentration of cryoprotectant used, which in turn provides an increased toxicity to the vitrification solution. However, a better understanding of the physical and biological principles of vitrification will lead to numerous and successful clinical applications of this technique. As of today, all developmental stages of human embryos cultured in vitro have been successfully vitrified and warmed. Cryopreservation techniques remain one of the key stones in the overall picture of clinical infertility treatment. Different embryonic stages will continue to be cryopreserved for different reasons whether practical, political or personal. Today, slow freezing technology still has the longest clinical track record, and greater ‘comfort level’ amongst embryologists. However, vitrification with its increased clinical application is showing a trend of greater consistency and better outcomes. When (not if!) IVF programs overcome the fear of the ‘unknown’, and take on the challenge of a short learning curve with vitrification, then will vitrification become a clinical standard for human embryo cryopreservation.