FUJIFILM
Value from Innovation

IrvineScientific

It's All About the Base: From Culture to Vitrification

H. Nadir Ciray, PhD, Research & Development Director, ART, FUJIFILM Irvine Scientific, Santa Ana, CA 92705

Introduction

The base is a vital component of media used for the vitrification and warming of embryos and oocytes, contributing to the viability and health of the biological materials in combination with other compounds that constitute the formulation. In this article, we consider two base media formulations—the conventional Medium 199 (M199) and the novel Continuous Single Culture Medium (CSCM)—and focus on the value of embryo-friendly base media during cryopreservation, which is inherently known to introduce stress to the oocytes and embryos.

The conventional base medium: M199

Many vitrification kits feature M199 base, a tried-and-true media solution in use since the 1990s. M199 was developed in 1950[1] with the purpose of producing a chemically defined medium to be used as a substitute for embryo extract (which was less than ideal due to inconsistencies between batches) and homemade horse serum (users needed to have their own horses to manufacture it).[2] M199 was later designed using combinations of amino acids, vitamins, and other factors to promote continuous growth of primary chick embryo heart and fibroblast cells without serum supplementation.[2]

The medium also contained unique components in comparison with other classical media, such as adenine, adenosine, hypoxanthine, thymine, and additional vitamins. Antioxidants such as glutathione and L-ascorbic acid that protect cells and embryos from oxidative stress were also included in the medium, supporting its role as an effective choice for vitrification and making M199 one of the key components of vitrification protocols as we know them. M199 is also used extensively in virology and vaccine production, as well as the tissue culture supply industry.

Collectively, M199 has a long-standing history of satisfactory results for culturing of embryos and oocytes. However, it was ultimately created for chick fibroblasts and there was room for improvement, opening the door for the transition of human embryo-specific Continuous Single Culture Medium (CSCM) in vitrification.

The novel base medium with continuity: CSCM

The new vitrification media from FUJIFILM Irvine Scientific, Vit Kit-NX, utilizes Continuous Single Culture Medium (CSCM) as the backbone medium. The purpose of this development is moving towards standardization of the base media used for various laboratory procedures for gamete and embryo handling during assisted reproduction technologies. This consistency of base media reduces stress upon embryos, which has been shown to result in reduced blastocyst utilization rate[3] due to the alteration of ingredients in the culture media composition.

CSCM follows a one-step culturing protocol that allows fertilized oocytes to reach the blastocyst stage without media change. A study conducted at Shady Grove Fertility Reproductive Science Center (Figure 1) showed that an uninterrupted culture system utilizing CSCM outperformed a competitor's two-step culture media system in regards to blastocyst development and implantation potential.[4]

Analogous to the “let the embryo choose” principle, this single step culture medium provides the embryo with the appropriate concentration of components from zygote to the blastocyst stage.

Through the elimination of media changes, which may induce variation in culture constituent concentrations, osmolarity, temperature, and pH fluctuations, embryo stress is even further reduced. Overall, CSCM helped to simplify embryo culture protocols and, as the base medium of Vit Kit-NX, to provide a simpler way to handle the culturing and vitrification of embryos.

Vit Kit-NX: Our latest advancement in vitrification media

Transition to Vit Kit-NX at the laboratory is seamless. Data shown by Aspire Houston Fertility Institute demonstrated that the Vit Kit-NX suite achieved high implantation and pregnancy rates equivalent, if not better than the original Vit Kit suite (Figure 2). (Case Study: Vit Kit-NX Suite Demonstrates High Implantation and Pregnancy Rates Equivalent to Vit Kit Suite. February 2022. FUJIFILM Irvine Scientific.)

Continuing with the superior results of CSCM, the Vit Kit-NX suite is now part of the FUJIFILM Irvine Scientific vitrification product offerings. Integration of the CSCM base allows for a seamless transition from the CSCM culture system into vitrification solutions, as embryos have already been acclimated to the base formulation during culture with our CSCM or CSCM-NX media.

Founded on a trusted formula and retaining the key benefits of the original FUJIFILM Irvine Scientific vitrification media, the addition of CSCM as the backbone delivers a consistent environment throughout the in vitro embryo cycle. Additionally, a dual-buffering system with both HEPES and MOPS provides a more secure pH environment for oocytes and embryos during vitrification. Furthermore, the addition of trehalose, a non-permeating cryoprotectant, supports a higher glass transition temperature and provides cell membrane stabilization, making this combination a superior choice for the embryo culture to vitrification process.

Conclusion: Less stress, higher success during cryopreservation

For IVF laboratories looking to replace M199 within their culture and cryopreservation processes, CSCM offers a solution that is designed specifically for human embryos and oocytes to provide the essentials of what an embryo needs across all developmental stages. By building on the benefits of current IVF media and tailoring it specifically for the vitrification process, the formulation of Vit Kit-NX is targeted to support oocytes and embryos, as well as create a steady, continuous system. Paired with vitrification and warming kits within the Vit Kit-NX suite, a continuous system is realized, resulting in less stress for embryos, oocytes, and clinicians alike.

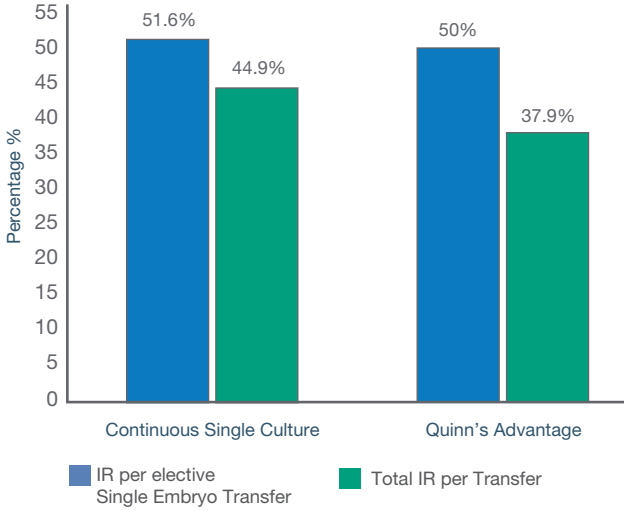

Figure 1. Comparison of Implantation Rates (IR) according to embryo culture media.[4]

Embryo Performance	Before	Cross-Warming	After
	Vit Kit - Freeze & Vit Kit - Thaw	Vit Kit - Freeze & Vit Kit - Warm NX	Vit Kit - Freeze NX & Vit Kit - Warm NX
Total Warmed:	2,343	310	3.202
Survival Rate:	97.7%	99.2%	98.5%
Single Embryo Transfer Rate:	78.4%	81.9%	87.5%
Implantation Rate:	49.6%	54.0%	53.5%
Clinical Pregnancy Rate:	56.0%	60.0%	57.4%

Figure 2. Strong performance of embryos vitrified and warmed with new vitrification media: Vit Kit – Freeze NX and Vit Kit – Warm NX.

References

1. Morgan JF et al. Nutrition of animal cells in tissue culture; initial studies on a synthetic medium. *Proc Soc Exp Biol Med.* 1950 Jan;73(1):1-8.
2. Morton HJ. Joseph F. Morgan: the man and his contributions. *In Vitro*. 1978;14(1):3-10. doi:10.1007/BF02618169
3. Gardner DK, Kelly RL. Impact of the IVF laboratory environment on human preimplantation embryo phenotype. *J Dev Orig Health Dis.* 2017; 8:418-435.
4. VerMilyea MD, Graham JR, Tucker MJ. Embryo Choice– A continuous single step culture medium protocol that provides greater embryo implantation potential whilst benefiting laboratory practices. Shady Grove Fertility Reproductive Science Center, Rockville, MD, USA.

FUJIFILM
Value from Innovation

IrvineScientific
www.irvinesci.com