Hello, "ICE Cubes" is the official e-newsletter of Innovative Cryo Enterprises I.C.E. I am publishing this to provide an ongoing connection to current users and others interested in cryopreservation. This e-letter will be published randomly, possibly on a bi-yearly basis, or as important news comes up. This e-letter will provide information and advice on all aspects of cryopreservation in the reproductive field. Thank you for your support!

**Did You Know???

**ICE has a website!** We can be found on the internet at: [www.icevitrification.com](http://www.icevitrification.com). We developed the site to be an information resource for cryopreservation. There is a wide variety of information on the site, from product information, protocols, to published articles and general information regarding cryopreservation and cryobiology. One main feature on the site is the Help Desk, where commonly asked questions and problem areas are discussed. As we learn more about cryopreservation/vitrification and what works and what people have difficulties with, we can disseminate this information to everyone in order to help clinics have increased success with whatever cryopreservation system they are using. It was our goal to provide an information resource anyone and everyone could use, rather than just a product website. Please let us know what you think of the site and how we can improve it.

**NEWS!**

**I.C.E. ADDS HYALURON TO VITRIFICATION SOLUTIONS**

I.C.E. constantly strives to produce and support the best vitrification products available today. Therefore I.C.E. has developed a new and improved blastocyst vitrification product containing hyaluron (HA). HA is a glycosaminoglycan (GAG) that has been found to improve post-thaw survival of animal and human embryos when added to vitrification solutions. I.C.E. began testing the addition of HA in its blastocyst vitrification media over a year ago and has had very promising results. In addition, other commercial media companies, including Vitrolife, are adding HA to embryo culture and vitrification products. The addition of a large macromolecule like HA offers dual support by increasing both the viscosity of the
vitrification solution (to a small degree) and supplemental cellular adhesion/membrane stabilization properties of the vitrification solution.

In a recent poll, most clinics using ICE blastocyst vitrification media have pregnancy rates with vitrified blastocysts near or slightly higher than with fresh blastocysts. The product has been widely successful as it is, with an estimated >1000 babies born worldwide. We hope that the addition of hyaluron to ICE blastocyst vitrification media will improve upon the success already obtained.

I.C.E. began testing the addition of HA in its blastocyst vitrification media over a year ago. Several clinics have tested the new media and found similar survival rates, whereas others have found improved results. Schiewe et al., 2013 ASRM, showed that human blastocysts undergoing trophectoderm biopsy survived vitrification in HA supplemented ICE media at a high rate (>99%; n=30). Implantation and pregnancy rates were 87.5%, 94.1% with HA, and 72.0%, 84.6% without HA, respectively. One goal is to move towards single embryo transfer. More single embryo transfers were performed in the HA group on average (1.33 HA vs. 1.67 no HA; embryos transferred per patient). Even though fewer embryos were transferred in the HA group the pregnancy rate was still slightly higher. Furthermore, all single euploid blastocyst transfers done in the study resulted in a pregnancy (17 of 30). He concluded that, in their experience, blastocysts vitrified in ICE vitrification media containing HA remained unchanged from their fresh state. It remains to be seen whether the trend of higher implantation and pregnancy rates will exist with greater numbers, however, the initial results are promising. No deleterious effects from the addition of HA have been reported.

Hyaluronan (also called hyaluronic acid or hyaluronate or HA) is an anionic, nonsulfated glycosaminoglycan that is distributed widely throughout connective, epithelial, and neural tissues. It is unique among glycosaminoglycans in that it is nonsulfated, forms in the plasma membrane instead of the Golgi, and can be very large, with its molecular weight often reaching the millions. HA is a polymer of disaccharides, themselves composed of D-glucuronic acid and D-N-acetylglucosamine, linked via alternating β-1,4 and β-1,3 glycosidic bonds. Hyaluronan can be 25,000 disaccharide repeats in length. Polymers of hyaluronan can range in size from 5,000 to 20,000,000 Da in vivo.

The main cell surface receptor for HA is CD44. CD44 is widely distributed throughout the body, and the formal demonstration of HA-CD44 binding was proposed by Aruffo et al. in 1990. CD44 mediates cell interaction with HA and the binding of the two functions as an important part in various physiologic events, such as cell aggregation, migration, proliferation and activation; cell to cell and cell–substrate adhesion. One of the chief components of the extracellular matrix, hyaluronan contributes significantly to cell proliferation and migration, and may also be involved in the progression of some malignant tumors.

HA has been used in attempts to treat osteoarthritis of the knee via injecting it into the joint. HA may also be used postoperatively to induce tissue healing, notably after cataract surgery. Current models of wound healing propose the larger polymers of hyaluronic acid appear in the early stages of healing to physically make room for white blood cells, which mediate the immune response. HA has also been used in the synthesis of biological scaffolds for wound-healing applications. These scaffolds typically have proteins such as fibronectin attached to the hyaluronan to facilitate cell migration into the wound.
Glycosaminoglycans are found at high concentrations in the fluid of the female reproductive tract of several mammalian species, including human. HA increases in concentration at the time of implantation and embryos have the CD44 receptor for HA from the oocyte stage through the blastocyst stage. HA also plays a role in sperm motility. (Gardner et al., 1999)

Gardner et al., 1999, showed that HA promoted embryo development to the blastocyst stage in vitro, and significantly increased implantation and fetal development, when supplemented during embryo transfer. Addition of HA to the culture medium containing either BSA or recombinant albumin also increased the ability of blastocysts to survive cryopreservation. Inclusion of recombinant albumin and hyaluronan in culture media facilitated the development of physiological defined culture conditions (Lane et al., 2003). HA has also been shown to improve the development of in vitro matured and fertilized (IVM/IVF) bovine embryos to the blastocyst stage (Furnus et al., 1998). Numerous other reports of the positive effect of HA in embryo culture, and more specifically during transfer, led to the creation of EmbryoGlue, a collaboration between Vitrolife and David Gardner. More support for the beneficial effect of HA during embryo transfer was given in 2007 by Friedler et al. This article contains numerous other references that also support the role of HA in embryogenesis.

In 2003 Gardner et al. showed that HA might also have a positive effect on cryopreserved embryos. Others proposed that, like some sugars, HA cryoprotects by providing substitute structure-stabilizing H-bonds. Block et al., 2008 showed that addition of hyaluronan to embryo culture enhanced blastocyst yield, improved survival following vitrification, and enhanced the post-transfer survival of fresh morula and blastocyst bovine embryos.

Vitrolife was so impressed at the beneficial effects of HA on pre- and post- implantation embryo development that it not only developed EmbryoGlue, but supplements its entire line (or the majority of it) of media with HA, including its cryopreservation products.

Selected References: (These articles reference many others supporting the role of HA)
Gardner DK, Rodrigez-Martinez H, Lane M. Fetal development after transfer is increased by replacing protein with the glycosaminoglycan hyaluronan for mouse embryo culture and transfer. Human Reprod. 1999: 14, 2575-2580.
Lane M, Maybach JM, Hooper K, Hasler JF, Gardner DK, Mol Reprod Dev. 2003 Jan;64(1):70-8. Cryo-survival and development of bovine blastocysts are enhanced by culture with recombinant albumin and hyaluronan.
Peer D, Florentin A, Margalit R. Hyaluronan is a key component in cryoprotection and formulation of targeted unilamellar liposomes. Biochim Biophys Acta. 2003 May 2;1612(1):76-82.
Good To Know!!!

Today, tomorrow, 20 years from now...
I.C.E. is here for you today, tomorrow, and even 20 years from now. I have been implementing a support system in the case that something should happen to me in the future (struck by lightning, trampled by heard of buffalo, etc.). Because all of you have numerous, even hundreds of embryos vitrified with the ICE vitrification system, it is our duty to ensure that these can be successfully devitrified as called for. This may be 10 years from now or longer. Additionally, clinics will need vitrification media for quite some time as well. We feel obligated to ensure that you will be always able to obtain vitrification and thawing media when you need it. Basically, I am organizing a highly trained backup team (Ph.D./HCLD embryologists) who will be able to continue to run I.C.E. should anything occur to me. Larger, reproductive companies have numerous employees and officials so that this is not an issue for them. Because I.C.E. is different, we are constantly adapting to the current needs, and future needs of IVF clinics.

I thank you for your continued support. We are always trying to improve this already successful system, in hopes of increasing survival and pregnancy rates so that all clinics using the system will achieve results similar to their fresh/nonfrozen embryo transfer rates. Many of you have already achieved this lofty goal and we are so glad that we could help. If you have any questions or comments about this newsletter or how to improve our system please contact me.

Best,
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