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Cryoport SRBT Webinar – A Vitrified Future in Cryobiology, A Tribute to the Pioneers
July 22, 2014

Question Log

Q: What technical improvements to vitrification are under consideration?

[Greg Fahy, PhD] *As noted during the webinar, efforts are being made to establish closed systems that are just as reliable and safe to use as open systems. One example is the recent SafeSpeed system of Dr. Ramon Risco in Saville. Another is the microSecure system of Mitch Schiewe. Both are user friendly and achieve cooling and warming rates sufficient to produce excellent results, but both need more field experience. Possibilities for improving the cryoprotectant solutions used for vitrification are also being explored.*

[Mitchel Schiewe, PhD] *There have been several device modifications that have occurred over the last decade, some to make a generational improvement in coloration (for ease of ID), and another to make it easier to extract an aseptic closed device. Other companies refuse to admit to their flaws, as changes in design cost them money, while others have been forced to in order to continue marketing. Another example was brought to our attention after the Webinar, of a new device in Europe (SafeSpeed) that in essence is a straw-like improvement of the Cryotip in terms of storage, labeling, and probably ease of loading without problematic bubble formation. Seems to have potential as a closed system, but still requires 2 different heat settings for sealing (which they seem to have accommodated with a custom sealer) and is not aseptic. In terms of solutions, there certainly is room for improvements, as very little experimentation has been done. Again companies concerned with improvements put R&D in place and modify their product. A couple years ago I had sodium hyaluronate (HA) added to my VS and potential improvements were gained. Although I only have a retrospective comparison (ASRM 2013; PCRS 2014), the addition of HA simply made sense (increases viscosity and potentially enhances membrane stability). Needless-to-say, there is room for improvements, that is why I took multiple QC factors into consideration when developing microSecure VTF.*

Q: For oocyte freezing, when oocyte is in equilibration solution, you wait for oocyte to recover or set a certain number of minutes until go to vitrification solution?

[Mitchel Schiewe, PhD] *As a purist from a cryobiology perspective, I have conceptual problems merging droplets to mix and concentrate a solution and to not use standardized intervals. Although I still prefer having a distinct intermediate solution (VS2) between the equilibration and final VS3, I now see the potential merit of variable equilibration times in VS1. In our limited experience in a small funded controlled trial, our first 10 donors produced: 95+% survival, 80+%2PNs, 8 preg, 6 live births; then the next (final) 5 donors had reduced survival (82-85%), similar fertilization and only 1 live birth. So, overall we had a comparably good live birth outcomes (7/15, 47%) similar to reported open systems, but we had 4 donors with real developmental incompetence issues which we believe were related to cytoplasmic maturation issues. We have recently initiated an IRB approved study to look at this,*

however a good friend at ESHRE (Debra Gook) pointed out that I may be over thinking the issue and the problem. That is, "it could simply be a maturation issue which has affected the membrane permability, requiring those eggs from those problem patients to need a longer equilibration time" in VS1 until they fully equilibrate. So, we have adopted a variable exposure time to the first solution, most likely depending more on the patient (and their COHstimulation/trigger) not the individual eggs, again in our limited experience.

Q: Does the use of DMSO in vitrification media concern you in any way?

[Mitchel Schiewe, PhD] *It is unclear to me where the dogma behind DMSO being unsafe really came from historically. As we all know, it has been used in the tissue culture/cell storage industry for decades, and even SF mouse embryos up to the mid-1980's. I am unaware of any confirmation of its potential mutagenic or teratogenic effects. It definitely has a higher permeation rate than the glycols > glycerol, thus there has been concern of cytotoxicity, but the short exposure intervals and combined low concentrations used in VTF would pretty much negate that concern. After all, toxicity is directly equated to [chemical organic contaminant-COC] x time of exposure. A recent study revealed: In a recent toxicity study, DMSO was actually shown to not exert any genotoxicity, whereas EG and PPG exhibited adverse DNA effects over extreme experimental conditions (24 h exposure / [varying]). (Aye et al., 2010)*

Q: Could laboratory animals (guinea pig) used for vitrification of oocytes?

[Greg Fahy, PhD] *Sure. There is no reason to think oocyte vitrification cannot be achieved in all mammalian species, although some are certainly easier than others. Many species have already been vitrified successfully.*

[Mitchel Schiewe, PhD] *Certainly, it could be a useful model, depending on the experimental application. Due to species variation in the lipid content of egg/blastomere cytoplasm, as well as membrane permeability potential, there may not be a direct correlation. However, in my experience I believe it is safe to say that mouse, and ironically (or fortunately) human, eggs and embryos are amongst the easiest to cryopreserve and culture. So if you can master a more challenging model, that experience should translate well to human application, dilution and elution intervals notwithstanding.*

Q: What were the multiple rates after oocyte vitrification?

[Zsolt Peter Nagy, PhD] *Oocyte vitrification in itself does not impact multiple rates. Just like when using fresh oocytes, some of the main variables that influence multiple rates are 1) patient parameters (age, medical history); 2) Embryo potential/viability; and 3) Number (and quality) of embryos transferred.*

Q: Mitch, are there not safety issues unrelated to infectious diseases? Open systems are not in direct contact with the tissue and this may result in inadvertent warming where if there was liquid nitrogen there instead of air it would be avoided.

[Mitchel Schiewe, PhD] *Not sure I understand the question. Open vs closed device consideration or LN vapor vs liquid. In terms of LN2, I believe most Embryologists feel most comfortable storing, handling and shipping embryos that have been in liquid. If for no other reason, cane-globlet contained devices retain liquid LN in their mini-storage environment making it completely safe to move a Patient cane with VTF devices from one tank to another or to a dewar flask or dry shipping tank. If shipping and then receiving it is always advisable to refill to tank with LN2 to insure the globets are refilled before removing the canes. I, for one, am not an advocate of LN2 sterilization/filtration as it raises safety concerns (that would not have existed) and it is expensive considering the LN vaporization loss. Finally, most labs use traditional <40L LN2 storage tanks, so to convert to Larger LN2 vapor tank is a big transition. Although I do use such a tank to temporary sperm freezing and storage at the California Cryobank, I feel much more comfortable with eggs and embryos stored in liquid LN2. Now, if you are comparing open to closed in terms of tissue exposure, both devices are in direct contact with the tissue they are vitrifying. The difference is that open system expose the VS and its tissue/cells directly to any liquid LN surrounding, with the possibility of charged contaminants adhering to it. Just because NO confirmed cases of viral cross-contamination have occurred, doesn't mean it's not potentially happening. In addition, we have recently shown (Zozula et al., AAB, 2014) in an ongoing study, that in our aseptic closed device, it can be "accidentally" removed for up to a minute without loss of the contents (100% recovery) and only a slight decline (10-20% at 0h and 24h, respectively) in viability upon reVTF. The null hypothesis being that this would not be true for an open system device. My personal opinion is that any system that is prone to not have 100% recovery due to device or technical variation is indeed seriously flawed, open or closed, and should not be marketed or FDA approved.*

Q: In case of slow DMSO frozen embryos, can we warm then using the same protocol for devitrification?

[Mitchel Schiewe, PhD] *If you are simply talking about DMSO-treated embryos frozen in a programmable unit at a "slow" rate <1.0C/min and directly plunged into LN2 at -30 to-38C, then standard rapid warming in a 32-37C water bath does apply to minimize intracellular devitrification. Whereas, slow frozen to -80C before plunge creates a much more dehydrated cellular environment not prone to deVIT at a slow warming rate. But slow warming (<100C/min) is necessary to reduce the injurious effects of osmotic over-swelling and lysis. In either case, the extracellular solution does crystalize during cooling and warming.*

Q: **Regarding the contamination due to the liquid nitrogen, which is better: Are the vapor system**

tanks more convenient and suitable than the liquid tanks for storage of the oocytes and blastocysts?

[Greg Fahy, PhD] *Vapor has theoretical advantages of avoiding contact with pathogens in the liquid phase, but has potential disadvantages of thermal instability. The latter can be addressed in several says, including using a system of isothermal vapor storage developed by 21st Century Medicine.*

[Mitchel Schiewe, PhD] ***S****ee Safety Issues question above.*