Innovative Cryo Enterprises L.L.C.

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I.C.E. CUBES #4

Technical information to help improve your cryopreservation practice

Hello, "ICE Cubes" is the official technical 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. You can also check out our website for even more information and past newsletters at: www.icevitrification.com. Thank you for your support!

Meetings!

I.C.E. will be at ASRM this year. ASRM October 18-22, 2014. There is a poster presentation on Oocyte vitrification with the ICE system. Results are shown by one of our clinics using the system.

Tips and Tricks!!!

Zygote Vitrification

There have been several reports, using various vitrification systems, of cleavage arrest and/or poor cleavage of thawed zygotes. Although some clinics have not had these issues, others have had conflicting reports where cleavage would be great in one case and the next it would be poor. The cause of this may be related more to the zygotes than to the actual freezing protocol. Around 18-20 hours post retrieval (and after ISCI or IVF) fertilized oocytes or 2PN's will exhibit pronuclei aligning with nucleoli scattered within the nucleolus. After another hour or so, these nucleoli start lining up in a more orderly manner. It is thought that it is better to freeze the 2PN embryos during 18-19 hours post retrieval when the nucleoli are scattered, than to freeze them later at 21-24 hours post retrieval when the nucleoli are lined up. Because of the complexity of syngamy and the processes that occur prior to the first cleavage, it may be disruptive and damaging to stress the cells by cryopreserving them during this period in their development. If anyone knows of other important points to consider when freezing 2PN zygotes, please contact us.

Very Important!

FET Transfers: Stimulation and Patient Preparation

Over the past few years there have been a number of clinics that have been concerned with optimizing their FET pregnancy rates. Pregnancy rates should be within 5% of pregnancy rates with fresh embryos; and in many cases the rates are higher for the frozen. Additionally, biochemical pregnancy rates should be the same or lower than fresh rates. Recent results (located on our website www.icevitrification.com) from a collection of clinics using the ICE Blastocyst vitrification system show that clinical pregnancy rates are similar between fresh and frozen transfers. However, some clinics have had issues with either lower than normal FET pregnancy rates and/or higher than normal biochemical pregnancy rates. We have tried to assist these clinics and in most cases have made significant progress.

Our experience has shown that the ICE vitrification system and protocol is not the source of low pregnancy rates and high biochemical rates. Modifying patient stimulation and progesterone supplementation regimes can significantly improve FET outcomes. Below are some of the changes that have been made at several clinics to alleviate the issue. If you are having a similar problem with lower than expected pregnancy rates with FET, please consider the pointers below and contact us for more information.

Timing of Progesterone Administration:

FET rates can be affected by the number of days and type of progesterone before embryo transfer. The idea here is to not advance the endometrium using P4 (especially Crinone and other vaginal progesterone) and close the window of implantation too early. Some clinics may put their patients on P4 for 5 or 6 days prior to the thaw.

It is important to note whether the patient starts progesterone in oil (PIO) vs. vaginal progesterone (like Crinone). It is thought that PIO takes longer (possibly 12-24 hours) to have an effect on the uterus, whereas vaginal has more of an immediate effect on the endometrium because it goes straight to the uterus. The dose of P4 may vary depending on patient response. You may need to vary the timing of embryo transfer based on the route of P4 administration. When using vaginal P4 some clinics perform the embryo transfer on day 5 or 5.5. However, it is widely suggested to do the thaw and transfer on D6 of P4 IM administration.

Type of Progesterone:

It is not recommended to use Crinone or a P4 suppository ONLY delivery system: 1) it makes for messy transfers and 2) they simply do not provide the consistent luteal support of I.M. injections. IM P4 is a pain, but it insures the correct delivery of P4 to the body Many centers alternate or combine IM P4 with vaginal progesterone with good results. We have heard from various clinics that doctors that allow patients to dictate their preference as vaginal progesterone only for luteal support have more variable outcomes and increased loss rates.

Embryo Quality:

Other than proper uterine preparation it is necessary to have good blastocyst quality pre- and post-thaw. Your vitrification criteria for blastocysts should be 4 BB or better. Of course this depends upon the patient, and sometimes we need to freeze everything because of age or other reasons. We need to consider the cycle that led to the embryos. It is necessary to document these items and the quality of the cohort from which the embryos were derived. Furthermore, are the conditions in your lab good and do you normally culture high quality blastocysts? Do many of your fresh transfers become pregnant? Are you doing Single ET routinely? In most cases the embryo quality before and after vitrification is good and the vitrification protocol is typically not the cause of the failed pregnancy or biochemical pregnancy.

Several labs have revised their P4 regime and have found significant increases in clinical pregnancy and delivery rates. If you suspect a similar issue in your clinic, please consider the above suggestions and contact us for more information.

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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