

The Cryotec Method:

The New Strategy That Makes 100% Survival Rate Possible

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REPROLIFE

At first, clinical application of vitrification preservation was considered impossible, which fell behind remarkably in the effectiveness and safety of preservation compared to the slow freezing method. However since the 1990's, efforts have aimed to lower negative effects of vitrification preservation, i.e. physical damage due to a cryoprotective agents of high concentration. Based on the vitrification theory of aqueous solutions, direct contact with coolant (conversion from closed to open system) as well as higher cooling speed rate due to minimization of samples, resulted in reduction of CPA concentration. With these findings was established a protocol that attains roughly 90% survival after preservation in any animal species.

In humans, the presenter developed the ultra-rapid cooling vitrification method that aimed for 95% survival rate based on the method for bovine blastocysts (The Cryotop Method: 1999). As for oocytes were considered difficult to vitrify, the equilibration method was improved to raise the survival rate (Step-wise Method: 2002). To reduce the cost even more, the solution recipe was improved by reinvestigating the type and concentration of additives, and the method was improved to attain the same survival rate even with low concentration, which was spread as the standard method throughout the world with Japan being the center.

For the past 15 years, due to vast clinical results exceeding 2,500,000 cases in over 75 countries, the vitrification method of open system developed by the presenter has been able to make >90% of human oocytes and embryos survive after vitrification, and besides it being extremely effective clinically, it had also proved that virus infection through liquid nitrogen did not actually exist.

The last remaining challenge was to rescue valuable oocytes and embryos that still had led to death, and making improvements to a noninvasive vitrification method that makes saving possible for women in true difficulty and pain, from low-grade oocytes/embryos in older patients to oocytes in women with cancer.

The strategy of 100% survival vitrification method consisted of decreasing cell injury due to osmotic pressure changes, considered to be the main adverse factor of the vitrification preservation method. Deciding to eliminate the primary factor that prevented oocyte and embryonic 100% survival led to the current vitrification method.

In order to minimize the osmotic pressure failure, 1) converting to trehalose which has higher vitrification-forming ability in vitrification solution, 2) increased viscosity

in vitrification solution, and 3) minimizing the CPA concentration have been implemented.

In addition, on the cause of embryonic death with the current vitrification method, all negative factors were eliminated such as 1) ambiguity in indicators of VS equilibration completion, 2) loading difficulty leading to excessive VS equilibration or improper VS drop shape and size, 3) bubble formation at the time of warming, and 4) flat-bottom plate leading to improper dilution of slowness. Thereby, the noninvasive vitrification method with a new protocol using new solutions and plates took place.

This vitrification method was completed as the extremely effective and safe noninvasive vitrification method (The Cryotec Method: 2012) that attains approximately 100% survival rate after vitrification in oocytes/embryos derived from needless to say, general patients, and also in low-grade oocytes and embryos derived from older patients and cancer patients. Already >100,000 cases in 60 countries have been implemented especially in Europe and the U.S., and with this high survival rate and excellent clinical results, ordinary methods of advanced infertility facilities in the world are changing.