

OOCYTE VITRIFICATION: A USEFUL PERSPECTIVE IN ASSISTED REPRODUCTION

A. Cobo

Cryobiology Unit at IVI, Valencia, Spain

Since many years, oocyte cryopreservation has been a challenging matter in assisted reproduction technologies (ART). The usefulness of this strategy becomes very clear if we consider those women who need an option for fertility preservation, like survivors of malignant diseases who suffer the chronic adverse effects of their cancer treatment, including gonadal failure and infertility. For these women, the option to cryopreserve their oocytes and store them in oocyte banks to safeguarding their potential to become pregnant in the future would be extremely valuable (1). A reliable option for fertility preservation would be also quite welcome for many women, who due to a variety of reasons wish to delay the age at which they become pregnant.

Another group of women who may benefit from oocyte cryopreservation would be patients with ovarian failure for whom oocyte donation is required. The establishment of banks of donated oocytes would considerably simplify the logistics and means by which oocytes could be donated. These banks would make it easier to immediately provide oocytes that would be compatible to the couple, shortening or even eliminating the problem of long waiting lists. An additional advantage of oocyte banking is that oocytes could be effectively quarantined and the risk of transmission of infectious diseases could be considerably reduced.

Oocyte cryopreservation will also contribute to solve several clinical situations in ART like the absence of a partner semen sample the day of ovum pick up. On the other hand, the possibility of accumulating oocytes from two or three stimulation cycles is an option for poor responder patients, to ensure an adequate number of oocytes for submission to insemination (2). In these cases, to build up a bigger cohort would theoretically increase the chance of selecting the best embryos for transfer. Additionally, oocyte cryopreservation in cases in which the embryo transfer is not advisable due to the risk of suffering ovarian hyper-stimulation syndrome (OHSS), would be greatly useful. For different reasons, to efficiently cryopreserve oocytes before insemination should be preferable than the option of storage a high number of embryos, as usually occurs in cases of OHSS. Gamete cryopreservation would help to overcome the problem of storing a numerous embryos that in many cases are forsaken by the patients/couples, especially when a conception is achieved after the first cryo-transfer. Finally, oocyte cryopreservation constitutes a significant opportunity in countries in which embryo cryopreservation is prohibited by law.

In spite of the evident clinical applications, the methodology applied up to now, basically slow freezing, has been disappointing, with results that has not been always reproducible.

Lately, vitrification has proven to be a very useful tool for oocyte cryopreservation, giving excellent results regarding survival and clinical outcome (3). This ice-free cryopreservation method has been modified in order to optimize results in humans (4-6). For example, decreasing the sample volume to 1 μ l and use of carrier systems of minimum capacity raise the rate at which heat is conducted out of the sample so that the sample cools at a very high rate.

The latest approach to minimum volume vitrification is the Cryotop device (Kitazato Supply Co., Fujinomiya, Japan) that was designed by M. Kuwayama (5). In our experience the developmental capability of embryos obtained from vitrified oocytes using the Cryotop method, is not affected by the vitrification procedure, since fertilization, embryo cleavage, quality and clinical results are similar to those achieved with fresh oocytes (7). Additionally, clinical results are higher than those obtained with other available techniques to date.

According to these evidences, we have established the egg-banking system for our ovum donation program and currently many patients are taking advantage of this strategy in our centre. One of the most remarkable benefits of the egg-banking is the elimination of the long waiting lists. With this option, once endometrial preparation has been completed, the donation can take place. Accordingly, the average time of estradiol replacement for recipients of our egg bank is 12.3 ± 2.7 days, being significantly lower than the spent for patients waiting for the reception of fresh oocytes (32.7 ± 12.4 ; $p < 0.05$). As mentioned above the overall outcome of the egg-banking system using vitrified oocytes is highly satisfactory. Survival rate after warming is over 90% (95.1%). On the other hand, the potential of vitrified oocytes is consistently similar than the one attained for fresh ones: fertilization rate (73.1% vs 74.6%), cleavage rate on day (95.5% vs. 97.6%), clinical pregnancy rate (51.4% vs. 52.6%) and implantation rate (39.2% vs. 41.7%) for vitrified and fresh oocytes respectively ($p < 0.05$). Regarding the population of infertile patients there is a specific aspect which deserves our special attention: the patient's age. We have analyzed the outcome of almost 1300 oocytes from 130 patients undergoing oocyte cryopreservation, according to maternal age. Our findings show that survival rates and clinical outcome of vitrified oocytes are impaired in older patients, may be indicating the survival and viability are oocyte-quality dependent. On the other hand, the clinical outcome observed in all groups of age has been similar to the one expected for fresh oocytes, indicating that the process of vitrification does not impair the potential of oocytes in order to generate competent embryos.

In cases of low response to controlled ovarian hyper-stimulation (COH) we have accumulated oocytes from 135 patients who underwent 304 COH cycles. In these cases, the mean number of embryos transferred is higher than those achieved in the group of patients who did not vitrificate their oocytes (2.0 ± 0.6 vs. 1.4 ± 0.3 respectively; $p < 0.05$), although the pregnancy rate (50.8%) was similar than the cumulative pregnancy rate/cycle observed in the fresh group (50.9%). Nonetheless 100% of the patients of the vitrification group were able to reach the 50.8% of PR, whereas with consecutive fresh cycles only 9.2% had this opportunity, because of the high percentage of abandonment of the treatment.

Nowadays oocyte cryopreservation by means of vitrification is providing a highly effective tool within ART, attaining similar outcomes than those obtained with fresh oocytes, thus allowing its application into clinical practice.

1. Cobo A, Domingo J, Pérez S, Crespo J, Remohi J, Pellicer A. Vitrification, an effective new approach to oocyte banking in healthy women, could be applied in cancer patients to preserve their fertility. *Clinical and Translational Oncology* 2008;10:268-73.
2. Cobo A, Bellver J, Domingo J, Perez S, Crespo J, Pellicer A *et al*. New options in assisted reproduction technology: the Cryotop method of oocyte vitrification. *Reprod Biomed Online* 2008;17:68-72.
3. Kuwayama M. Highly efficient vitrification for cryopreservation of human oocytes and embryos: the Cryotop method. *Theriogenology* 2007;67:73-80.
4. Liebermann J, Tucker MJ. Effect of carrier system on the yield of human oocytes and embryos as assessed by survival and developmental potential after vitrification. *Reproduction* 2002;124:483-9.
5. Kuwayama M, Vajta G, Kato O, Leibo SP. Highly efficient vitrification method for cryopreservation of human oocytes. *Reprod Biomed Online* 2005;11:300-8.
6. Vajta G, Nagy ZP. Are programmable freezers still needed in the embryo laboratory? Review on vitrification. *Reprod Biomed Online* 2006;12:779-96.
7. Cobo A, Kuwayama M, Perez S, Ruiz A, Pellicer A, Remohi J. Comparison of concomitant outcome achieved with fresh and cryopreserved donor oocytes vitrified by the Cryotop method. *Fertil Steril* 2008;89:1657-64.