A comparison of two different vitrification methods for cryopreservation of mature human oocytes

S. Kagalwala, G. Gandhi, G. Allahbadia, M. Kuwayama, A. Allahbadia, V. Chipkar, A. Khatoon, R. Ramani, M. Madne, S. Ailsule
Rotunda - The Centre for Human Reproduction, Mumbai, India.

Introduction
Cryopreservation of oocytes serves as a valuable tool in human assisted reproductive techniques. It would permit cancer patients to preserve their reproductive capacity before undergoing potentially sterilizing cancer treatment. Oocyte cryopreservation can be helpful to women with other medical conditions leading to premature menopause, and healthy women wanting to delay childbearing for a variety of reasons. Additional group of women who may benefit from oocyte cryopreservation would be patients with ovarian failure for whom donor oocytes are required.

Stimulation Protocol for oocyte donors
Controlled stimulation protocol using biosimilair recombinant FSH (Foligraf,BSVL, India) along with Buserelin (Busag, Zydus Gynova, India) was used.

Protocols for oocyte vitrification
Vitrification of oocytes was carried out within two hours of retrieval. Two vitrification methods Cryotop (Kitazato, Japan) and Cryotech (Cryotech, Japan) were used.

Vitrification of oocytes using Cryotop method (Kitazato, Japan)
Oocytes were initially equilibrated in Basal solution (BS) and gradual increase in Equilibration solution (ES) concentration was achieved in 15 minutes. Subsequently oocytes were washed thrice in Vitrification solution (VS) so as to completely remove any traces of ES. The oocytes were then transferred to second well of VS, where they were washed twice and transferred with minimal volume on the surface of the cryotop carrier. The cryotop was plunged into liquid nitrogen, and kept inside a protective straw-cup prior to cryo-storage within liquid nitrogen.

Vitrification of oocytes using Cryotech method (Cryotech, Japan)
Oocytes were vitrified by a two step protocol with Cryotech Vitrification Kit using cryotopes as the carrier device. Oocytes were initially equilibrated in Equilibration solution (ES) for 15 mins, and subsequently moved in Vitrification solution (VS). In VS it is washed thoroughly till ES is completely displaced by VS. The oocytes were later washed in second well of VS, and loaded on cryotec carrier. The cryotec is plunged into liquid nitrogen and capped and stored in the liquid nitrogen.

Warming of Oocytes
The protocol for warming the oocytes was the same in both the methods. The oocytes were warmed using a four-step dilution procedure. Briefly, the carrier device containing the oocytes was removed from the protective straw-cup and dipped into thawing solution (TS) at 37°C for equilibration. After 1 minute, the oocytes were placed in diluent solution (DS) for 3 minutes. Later, the oocytes were transferred to washing solution (WS) for 5 minutes, which was followed by a final wash in second well of WS. The warmed oocytes were incubated for 2hrs before intracytoplasmic sperm injection (ICSI).

ET in Recipient
Oocytes were transferred on day 3 after preparing the recipient endometrium with Estradiol Valerate tablets (Progynova, Zydus Healthcare, India).

Results
Total of 56 donor egg cycles were included in this study. The mean values for the donor age and recipient age were comparable. Total number of oocytes vitrified and warmed using Cryotech method was 275 and the total number of oocytes vitrified and warmed with Cryotop method was 336. The survival rate of oocytes warmed with Cryotech method was 97.17% and that with Cryotop method was 95.14%. The fertilization rates of the oocytes after carrying out intracytoplasmic injection (ICSI) were 90.72% and 86.15% in Cryotech and Cryotop groups respectively.

It was interesting to note that there was statistically significant (p<0.05) difference in the cleavage rates, 96.85% for the Cryotech group and 91.88% for the Cryotop group. Statistically significant (p<0.05) difference was also seen in the pregnancy rates, 54.83% for the Cryotech group and 40.57% for the Cryotop group.

Discussion
Recent improvements in the efficiency of oocyte cryopreservation by means of the vitrification method has allowed oocytes to be frozen for the establishment of donor oocyte banks. These oocytes can then be used for the fertility treatment for patients who may resort to an egg donation cycle in order to achieve conception.

The present study was an analysis to compare the efficiency of two different oocyte vitrification methods –The Cryotop and The Cryotech, by evaluating oocyte developmental competence post warming and clinical outcomes of the warmed cycles. The Cryotech method was found to have several advantages over the Cryotop Method. In the Cryotech media, sucrose is replaced by trehalose which overcomes the problem of endotoxins present in sucrose. The Cryotech media does not contain serum and synthetic substrate(SSS). Hence, there is no risk of serum derived virus contamination. There is a groove provided for holding the cryotop on the Cryotech vitrification plate, hence microscope focus remains the same while loading the oocyte on the cryotec. This leads to a great ease in handling. There is no blind space in wells, hence the chances of losing oocytes during washing is reduced to a great extent. The Cryo tec has a long and wide handle with enough space for labelling.

It is thought that all these advantages cumulatively add up to give a higher survival rate and better developmental potential when the oocytes are vitrified using the Cryotech system. The present study showed a higher survival rate with Cryotech though difference was not statistically significant. The analysis showed a statistically higher cleavage rate and pregnancy rate in the Cryotech group. This can be attributed to less trauma caused to the oocytes during vitrification and warming using the Cryotech media.

Cryotech vitrification is proved to be a highly effective method of oocyte vitrification and can be very successfully used for building donor oocyte banks as well as for fertility preservation for oncologic and non-oncologic reasons.