A comparision of two different vitrificatiom methods for cryopreservation of mature human oocytes

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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.

Oocytes are sensitive to chilling injury and are damaged when cooled slowly by standard freezing methods. The efficacy of oocyte cryopreservation has been dramatically enhanced with the introduction of vitrification, which provides outcomes similar to those accomplished with fresh oocytes and opens up a wide range of applications. After the first report of a successful pregnancy using a frozen thawed oocyte in 1986, efforts have been made to develop an ideal oocyte cryopreservation method.

Dr. Masashige Kuwayama developed the Cryotop method in the year 2000. The cryotop device consists of a fine, transparent polypropylene film attached to a plastic handle and is equipped with a cover straw. The cryotech method is the most improved and latest method of vitrification developed by Dr. Kuwayama. The Cryotech method differs from all the earlier methods of vitrification in terms of the constituents of its solutions as well as the design of the vitri/ warm plates and the carrier device.

In our current study, we have applied the Cryotop and the Cryotech method for vitrification in our oocyte donation program, assessing fertilization, cleavage, and embryo developmental parameters. This study allows us to critically evaluate the reliability of these vitrification methods.

Materials and Methods

This is a retrospective data analysis of donor egg -IVF cycles using vitrified oocytes from October 2010 to August 2012. The oocytes were vitrified using either the Cryotop or Cryotech vitrification method. A total of 611 mature oocytes were vitrified and 131 embryo transfer cycles were performed using the embryos created after fertilizing the warmed oocytes with ICSI.

Stimulation Protocol for oocyte donors
Controlled stimulation protocol using biosimilar 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 inserted into a protective straw-cap prior to cryo-storage within liquid nitrogen.

Vitrification of oocytes using Cryotec method (Cryotech, Japan)
Oocytes were vitrified by a two step protocol with Cryotech Vitrification Kit using cryotec 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-cap 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
Embryos 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.

Tables & Graphs

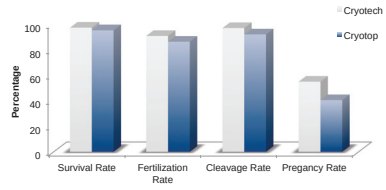
Table 1: Donor & recipients Characteristics

	Cryotech	Cryotop	p value
No. of donor cycles	26	30	
Mean Age of donors	25.3±2.8	24.8±2.4	NS
No. of recipient cycles	62	69	
Mean Age (±) of recipients	39.1±4.8	37.4±5.5	NS
No. of oocytes retreived	10.58±7.1	10.22±6.8	NS
Total No. of MII oocytes vitrified	275	336	
Mean no. of oocytes warmed per recipient	4.43±0.53	4.86±0.33	NS
Mean no.of oocytes for ICSI	4.30±0.58	4.62±0.51	NS

Table 2: Comparison of clinical outcome in frozen/ Warmed oocytes recipients cycles using cryotop and cryotech

	Cryotech	Cryotop	p value
No. of oocytes warmed	275	336	
No. of oocytes survived	267	319	
Survival Rate (%)	97.17	95.14	NS
No.of fertilized oocyte	240	274	
Fertilization Rate (%)	90.72	86.15	NS
No. of cleaved day 3 embryos	232	251	
Cleavage Rate (%)	96.85	91.88	P<0.05
Total no. of grade A & Grade B embryos	225	240	
Pregancy Rate (%)	54.83	40.57	P<0.05

Comparison between cryotech and cryotop methods of oocyte vitrification



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 must 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 serum substitute(SSS). Hence,there is no risk of serum derived virus contamination. There is a groove provided for holding the cryotec 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 Cryotec 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 this 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.