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An Update of Oocyte Vitrification: A Modification of Sucrose and Trehalose as Extracellular Cryoprotectant

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ABSTRACT

As well as the development of assisted reproductive technology (ART), as the current treatment of woman who failed in achieving pregnancy, the development of an advance vitrification method also grows rapidly. The successful of oocyte vitrification depends on the type and the concentration of cryoprotectant. This study was addressed to elaborate empirical evidence and recent studies of sucrose and trehalose as an extracellular CPA with the aim of achieving the success of oocyte vitrification. Several researchers in agreement that trehalose, as extracellular cryoprotectant, also has a role as intracellular cryoprotectant by microinjection with high survival rates as the outcome. Moreover, the combination of sucrose or trehalose as an extracellular cryoprotectant and others intracellular cryoprotectant have different survival rates which might occur because of the differences between the composition and concentration of sucrose or trehalose. The appropriate type and concentration of sugar as an extracellular cryoprotectant for oocyte cryopreservation are sucrose or trehalose in 0.5M concentration. Nevertheless, it requires further study to optimize oocyte vitrification process.

Keywords: Oocyte vitrification, Extracellular cryoprotectant, Sucrose, Trehalose.

INTRODUCTION

The renewed highlight of current research is concerning on human oocyte cryopreservation. Oocyte cryopreservation procedure could be one solution among infertile women when embryo cryopreservation is strictly forbidden. To date the ultimate goals of oocyte cryopreservation method development are enhancing survival rate of the oocyte, achieving pregnancy and improving implantation rate.[1] Oocyte cryopreservation could be conduct either by slow freezing or vitrification technique.[2] It is one of the advanced methods in maintaining female fertility where oocytes were obtained, freeze and stored.

Slow freezing methods require controlled decreasing of gradually temperature and dehydration of the cells to prevent intracellular ice forming. Yet the cell is remained to be deleterious due to the increase of electrolyte concentrations. The utilization of slow freezing method to human oocytes is

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resulting in the various type of injuries such as cell lysis, alteration of cytoskeleton and spindle microtubules, parthenogenetic activation, and polyploidy leading to the low success rate and poor development of blastocyst stage.[2] As an opposed, vitrification is an ultra-rapid freezing method by which a highly concentrated of cryoprotectant solidified during cooling without intracellular ice forming. Cryoprotectants are any salute of chemicals that used to protect or reduce the cell from damage risk during freezing and thawing, nevertheless, it could damage cells due to osmotic effects and chemical toxicity.[3]

Some parameters in achieving the success of cryopreservation are being concerned such as cooling rate, the type, and concentration of cryoprotectants which allow higher post-thaw recoveries.[4] The successful of vitrification depends on the type and the concentration of CPA which further also could be classified as extracellular and intracellular CPA.[5] To date, the conventional freezing solution which is frequently used in oocyte cryopreservation consists of non-permeating agents which is greater than 0.5 M such as trehalose and sucrose or permeating agents that is greater than 4M such as Ethylene Glycol (EG), Propanodiol (PrOH), Dimethyl sulfoxide (DMSO) or acetamide. Recent studies demonstrated that DMSO and ethylene glycol was the most effective intracellular CPA. In contrast, there were fewer studies conducted about extracellular CPA.[6] Therefore, this review aimed to elaborate recent studies of sucrose and trehalose as an extracellular CPA.

Sucrose and Trehalose as Extracellular Cryoprotectant Media

The success rate of human oocyte cryopreservation is remained low and unsatisfactory while exposure of liquid nitrogen to oocyte poses a biosafety risk during vitrification procedure. Until recently, numerous published data reported the development of reliable protocols and alternative approach to achieve vitrification success.[7] One of which is using sugar that known as a compound which used to survive from extreme drying and freezing by a variety of organisms.[8] Several groups elaborated studies on developing sugar-based preservation methods particularly using sucrose and trehalose as extracellular cryoprotectant media.

The utilization of sucrose and trehalose as media for vitrification of oocyte is demonstrated in table 1. (Table 1) Recent data perform not only single trehalose or sucrose but also the combination of both with another CPA, are being utilized either for a freezing method or vitrification. Eroglu[2] in 2002 presented trehalose has a critical role as an extracellular CPA, moreover, trehalose acts as intracellular CPA by microinjection, achieving 66% of survival rate up to -60^0 C of cooling temperature. Following up to previous research, Eroglu *et al.*,[9] assessed the freezing method using intracellular trehalose 0.15 M by microinjection combine with extracellular DMSO 0.5 M, outcoming 84% of mice oocyte survival rate. Findings demonstrated that trehalose allowed the used of lower concentration hence the toxicity due to cryomedium reduced. Nevertheless, microinjection technique which carried out is considered contribute to another oocyte damage.

As well as trehalose, to date, there was still no researches have been conducted in order to use single sucrose as the medium of cryopreservation. Combination of sucrose and another intracellular CPA agent such as EG, PrOH, and DMSO performed ranging of oocyte survival rates. Combination of sucrose and trehalose in the slow freezing method, by Borini *et al.*,[10] achieved 74.1% of oocyte survival rate. Another research by Bianchi *et al.*,[11] oocytes were cryopreserved with used 1.5mol/l propanediol and 0.3 mol/l sucrose, 22.9% showed a weak birefringence spindle signal, while 1.2% of oocyte displayed a high signal. Elaborated study in oocyte vitrification, either the combination of cryoprotectant or concentration was varied such as propylene glycol,[12] propanodiol and ethylene glycol,[13-16] resulting the survival rate range from 65% to 92.43%. It was considered that various factors may influence the outcome of vitrification method such as differences of composition and concentration of sucrose used. Moreover, the established research demonstrated that there was not appropriate sucrose concentration which performed optimum oocyte survival rate.

The previously researches were developed by using either freezing methods or vitrification with the utilization of single trehalose or combine to another CPA. The combination of trehalose

Table 1: Sucrose and trehalose as extracellular cryoprotectant media

Authors	Study design	Cryopreservation Protocols	Study Group	N	Control Group	N	Outcomes of study group
Eroglu *et al*., 2002 [2]	Randomization of sibling human oocytes	Slow freezing : Intracellular Trehalose 0.15 M + Extracellular Trehalose 0.5 M	Oocyte slow freezing	125	Fresh oocyte	33	Cryosurvival rate (66%)
Eroglu *et al*., 2009 [9]	Randomization of mice oocytes	Slow freezing : Extracellular Trehalose 0.15M + Intracellular DMSO 0.5M	Oocyte slow freezing	480	Fresh oocyte	N/A	Cryosurvival rate (84%)
Borini *et al*., 2006 [10]	Randomization of sibling human oocytes	Slow cooling: Sucrose 0.3M + Propanodiol 1.5M	Oocyte slow freezing	927	N/A	N/A	Cryosurvival rate (74.1%) Pregnancy rate (18%) Implantation rate (5.25)
Bianchi *et al*., 2012 [11]	Randomization of sibling human oocytes	Slow freezing : Propanediol 1.5 M + Sucrose 0.3 M	Oocyte slow freezing	443 cycles	N/A	N/A	Cryosurvival rate (71.8%) Fertilized rate (77.9%)
Emeka *et al*., 2015 [12]	Randomization of sheep oocytes	Vitrification: Propylene glycol 5 M + Sucrose 1.5 M	Oocyte vitrification	350	N/A	N/A	Cryosurvival rate (92.43%)
Zhang *et al*., 2017 [13]	Randomization of sibling human immature oocytes	Vitrification: Sucrose 0.5M + Propanodiol 7.5% + Ethylene glycol 7.5%	Oocyte vitrification	519	Fresh oocyte	291	Cryosurvival rate (84.9%)
Hurtt *et al*., 2000 [14]	Randomization of bovine oocytes	Vitrification: Ethylene glycol 2.5M + Sucrose 0.5 M	Oocyte vitrification	50	Fresh oocyte	50	Cryosurvival rate (70%)
Kuleshova *et al*., 1999 [15]	Randomization of sibling human immature oocytes	Vitrification: Ethylene glycol 0.5 M + Sucrose 20.54%	Oocyte vitrification	17	N/A	N/A	Cryosurvival rate (65%)

table 1 continues...

Rayos *et al.*, 1994 [16]	Randomization of mice oocytes	Vitrification: Ethylene glycol 3.0M + Sucrose 0.25M	Oocyte vitrification	527	Fresh oocyte	94	Cryosurvival rate (77.4%) Fertilized rate (84.5%)
Lee *et al.*, 2010 [17]	Randomization of mice oocytes	Slow freezing : Propanodiol 1.5M + Trehalose 0.5M	Oocyte vitrification	40	N/A	N/A	Cryosurvival rate (90%)
Zhang *et al.*, 2017 [13]	Randomization of sibling human immature oocytes	Vitrification: Trehalose 0.5M + Propanodiol 7.5% + Ethylene glycol 7.5%	Oocyte vitrification	519	Fresh oocyte	291	Cryosurvival rate (83.3%)
Rayos *et al.*, 1994 [16]	Randomization of mice oocytes	Vitrification: Ethylene glycol 3.0M + Trehalose 0.25M	Oocyte vitrification	527	Fresh oocyte	94	Cryosurvival rate (71.3%) Fertilized rate (86.1%)

among another cryoprotectant concentration was varied such as propanodiol.[13, 17] and ethylene glycol.[16] The results of the researches displayed ranging of survival rates from 71.3% up to 92.43%. It is suggested that various outcome of vitrification influenced by different concentration which used in the procedure. Recent studies performed that trehalose was more effective and safe as CPA than sucrose. Findings reported that trehalose was superior to another sugar on membrane stabilization. In addition, trehalose was the most effective sugar which has stabilize liposomes during drying and has special abilities in preserving dry and frozen biological materials.[18]

The major limitation of vitrification up forward is the deleterious effect of high concentration of cryoprotectant which capable to induce the glassy vitrified state. The approach could be used in order to enhance cryosurvival rate is by minimizing osmotic injury and cryoprotectant toxicity by reducing the concentration of cryoprotectant. Developing of traditional vitrification seems more complex and difficult to control besides the concentrations effect and due to the requirement of multiple loading steps and short exposure times to the high concentration of cryoprotectant.

Currently, the rapid development of an ultra-rapid vitrification for oocyte uses sucrose and trehalose as extracellular cryoprotectant with the technique used is similar to standard slow-freeze vitrification protocols. The undertaken study regarding the utilization of sugar as extracellular cryoprotectant performed good outcome, particularly in the cryosurvival rate. Notably, the sugar property, which impermeable towards mammalian cell membrane, represents a disadvantage regarding their utilization as an intracellular cryoprotectant. Hence, it is difficult to extrude intracellular sugars after cryopreservation. Nevertheless, intracellular sugar is possible being metabolized and discharged by exocytosis after several periods. Interestingly, findings reported that cell capable to tolerate intracellular sugar either trehalose or sucrose up to 0.2 M without interrupting cell functionality despite the effect toward human oocyte remain unknown. Therefore, further studies towards fertilization and oocyte development after cryopreservation have to be accomplished prior being used in the clinical application.[2]

The current development of extracellular sugar as cryoprotectant performs a novel promising of ultra-rapid vitrification. The extracellular sugar acts by drawing the water within the cell. The combination of extracellular sugar, impermeable agent, and permeating cryoprotectant prevents intracellular damage from ice crystal formation.[19] Several authors in agreement regarding sucrose and trehalose as cryoprotectant that exhibited a good result towards oocyte survival rate and development of post-thawing, pregnancy and implantation rate yet the results vary from bench to bench.

CONCLUSION

Technically oocyte vitrification is tough to carry out, due to the very viscous, small and concentrated volumes of solutions used where the oocyte should get handled for a very limited amount of time that is less than one minute during and before cryopreservation vitrification. Therefore, to attain the maximum oocyte rate of survival carried out by cryopreservation needs an advanced skill. In conclusion, there is still no appropriate type and concentration of sugar as the extracellular CPA for oocyte cryopreservation. Nevertheless, trehalose appears more superior to sucrose regarding the success of oocyte cryopreservation. It requires the continued study to optimizing the type and concentration of CPA for oocyte vitrification. The successful development of human oocyte cryopreservation will be of enormous benefit to its applications in the field.

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