CORE
Metadata, citation and similar papers at core.ac.uk
Provided by Jurnal Kesehatan Reproduksi

MICROVOLUME OF 0.1μL GAMA SLEEVED CRYOLOOPS FOR BLASTOCYST VITRIFICATION OF ASSISTED REPRODUCTIVE TECHNOLOGY PATIENTS

Ita Fauzia Hanoum[1,2], Arief Boediono[3], Mulyoto Pangestu[4,5], Dwi Haryadi[1], Shofwal Widad[1,2], Djaswadi Dasuki[1,2]

ABSTRAK

Latar Belakang: Prosedur embrio vitrifikasi menggunakan alat berupa *grid, straw* atau *cryoloop*. *Gama Sleeved cryoloop* dibuat dan dikembangkan di klinik Permata Hati. Untuk itu, dilakukan pengamatan keberhasilan prosedur vitrifikasi menggunakan *0.1μl Gama Sleeved cryoloop.*
Metode: Vitrifikasi dilakukan pada blastokis dengan kualitas baik yang diperoleh pada hari ke 5 setelah fertilisasi. Inform consent telah disampaikan sebelumnya kepada pasien program bayi tabung di Klinik Permata Hati. Prosedur dilakukan dengan menggunakan media *handling* (GMOPS Plus; Vitrolife) embrio diinkubasi selama 1 menit; (7.5% EG (v/v); 7.5% DMSO (v/v)) selama 2-3 menit, (15% EG (v/v); 15% DMSO v/v; 10 mg/ml Ficoll; 0.65 M Sucrosa) selama 30 detik pada suhu ruang sebelum kemudian diletakkan di dalam cryoloop, setelah itu secara cepat cryoloop yang berisi embrio dibenamkan ke dalam nitrogen cair. Sebelum dilakukan *embryo transfer* (ET), embrio dihangatkan dengan cara *two step technique* (sucrose 0.25M) selama 2 menit dan selama 3 menit (sucrose 0.125M).
Hasil: Sejumlah 97 blastokis divitrifikasi dan dihangatkan (67 pasien), dimana 91 blastokis berhasil ditransfer ke rahim ibu (93.8%). Blastokis yang tidak berhasil selamat dari prosedur penghangatan adalah blastokis dengan kerusakan lebih dari 50%. Diperoleh kehamilan klinis 43.3% sedangkan angka implantasi adalah 37.4%. Sampai saat ini, dilaporkan 20 kelahiran (23 bayi) dari program vitrifikasi menggunakan 0.1μl Gama Sleeved cryoloop, sementara 5 kehamilan masih berlangsung. Satu kehamilan dilaporkan gugur pada usia kehamilan yang masih sangat awal, dua keguguran pada usia kehamilan 12 minggu dan satu bayi lahir meninggal karena kelainan kongenital.
Kesimpulan: *0.1μl Gama Sleeved cryoloop* merupakan pilihan untuk digunakan sebagai alat vitrifikasi blastokis. Data awal yang kami sampaikan dan kelahiran bayi dari program tersebut memberikan harapan untuk kesuksesan program simpan beku embrio di klinik Permata Hati RSUP DR Sardjito Yogyakarta.

Kata kunci: kriopreservasi, blastokis, vitrifikasi

ABSTRACT

Background: Vitrification has been applied succesfully in human embryo using grid, straw and cryoloop. Gama Sleeved is a home made device develop at Permata Hati. We assessed the survival rate of human blastocyst vitrified in 0.1μl Gama Sleeved cryoloop as device.
Method: Excess good grade human D5 embryos were vitrified, upon a detailed informed consent. Embryos were hold in handling media (GMOPS Plus; Vitrolife) for 1 minute; (7.5% EG (v/v); 7.5% DMSO (v/v)) for 2-3 minutes, (15% EG (v/v); 15% DMSO v/v; 10 mg/ml Ficoll; 0.65 M Sucrosa) for 30 seconds at room temperature before inserted in

[1] Permata Hati Infertility Clinic RSUP DR Sardjito, Yogyakarta
[2] Div Reproductive Endocrinology and Fertility OBGYN Medical Faculty Gadjah Mada University, Yogyakarta
[3] Lab. Anatomi Embriologi FKH, Institut Teknologi Pertanian, Bogor
[4] EPRD- Dept. Obstetrics and Gynecology, Monash University, Monash Medical Center,Victoria, Melbourne
[5] Lab. Reproductive Physiology, Jenderal Soedirman University, Purwokerto
Correspondence address: + 62 274 518684; fax + 62 274 553575; email: *itafauzia@yahoo.com*

to the loops, then directly plunged into the liquid nitrogen. Prior to ET, embryos were warmed by two step technique in sucrose 0.25M for 2 min and 0.125M sucrosa for 3 min. Embryos were then cultured.

Results: Total of 97 vitrified warmed human blastocyst (67 patients) were used and 91 (93.8%) were transferred. Non-transferred blastocyst (6.2%) has more than 50% lyse. The clinical pregnancy rate was 43.9%. The implantation rate was 37.4%. Currently, 20 deliveries of 23 babies born from vitrified blastocyst using 0.1µl Gama Sleeved cryoloop, and another 5 ongoing pregnancy. So far there was 1 early pregnancy loss, 2 miscarriages at 12 weeks pregnancy, and one infant died due to a congenital anomaly.

Conclusion: 0.1µl Gama Sleeved cryoloop provides an excellent alternative to existing vitrification devices. These initial data and babies delivered from the program have been promising to a vitrification system in our own ART program.

Keywords: cryopreservation, blastocyst, vitrification

INTRODUCTION

The development of ovarian hyperstimulation protocols and culture system by sequential media has proven to improve pregnancy rates in ART and the number of embryos produced.[1] Consequence of increased number of transfering embryo will be high incidence of multiple gestation. Therefore, it has been proposed necessarily to limit the number of embryos to be transferred.[2,3] Transfering a single embryo or two would reduce the incidence of multiple gestations while maintaining the pregnancy rates[4]. Extended culture to blastocyst stage seems to be a usefull aproach since developmental arrest are frequently occur at earlier stages.[1,5]

In a situation of OHSS (Ovarian Hyper Stimulation Syndrome) or when there is an extreme difficulties in embryo replacement procedure, embryo cryopreservation would be an advantage. When a problem occurs, the embryos will be preserved. They can then be transferred in an elective cycle.

Slow freezing remains the most commonly used method for embryo preservation in ART laboratories around the world, but many studies have recently reported an increasing success of clinical results with vitrification.[6,7,8,9]

Important factor in achieving optimal result of vitrification procedures is cryoprotective agents (CPA).[10,11,12] Cryoprotectants are essential for the cryopreservation of cells. During vitrification, there is a practical limit of the procedures to attain cooling speed, and a biological limit of the cells on the concentration of cryoprotectant tolerated by cells. A critical concentration of cryoprotectant is required for vitrification. Variation in concentration can lead to either osmotic or chemical toxicity. Balance between maximizing the cooling rate and minimizing the cryoprotective concentration is important.

Another important factor to improve outcomes is device in order to accommodate embryos in such a way that the embryos will not lose and sustain their viability. Types of devices used for vitrification, including in human services application, there are hemi-straw, open pulled straw, microscopic grid, cryotop and cryoloop that have been investigated to increase the cooling and warming speed; thus, the CPA concentration can be reduced up to 25-35%.[7,6,13,14]

This present study reported our initial clinical data as well as live birth rate outcomes , with the 0.1µl Gama Sleeved cryoloop. The efficacy of the divice to preserve embryo at the blastocyst stage was analyzed following frozen embryo transfer (FET) program at Permata Hati Infertility Clinic-RSUP DR Sardjito.

METHOD

Blastocyst culture

Patients administerred for IVF program in our infertility clinic were agreed to vitrified some of their viable blastocyst upon completion of inform consent. All couples participating in the study signed and approved consent form prior to the method application. The mean age of the women was 33.1 years and range was from 28-39 years old.

Women were treated with GnRH agonist and hMG in either a long- or a short-treatment protocol. Oocytes were collected 36 hours after hCG administration using the vaginal ultrasound-guided procedure. The oocytes were inseminated by either conventional IVF or intracytoplasmic sperm injection. Fertilization was assessed 16-18 hours after insemination. Embryos having two pronuclei were washed well and cultured in groups of two to three in G1-Plus media (Vitrolife, Gothenburg, Sweden) in an atmosphere of 6% CO2, 5% O_2, and 89% N_2. After 48 hours of culture in G1-Plus, embryos were selected for embryo transfer procedure. Excess embryos were rinsed three times and transferred into G2-Plus medium (Vitrolife, Gothenburg, Sweden) for further 48–72 hours culture. On day 5 or day 6, embryos were assessed for their development into blastocysts. Good grade blastocysts were vitrified with the Gama Sleeved cryoloop.

Cryoloop

Gama Sleeved is a home made cryoloop. Handle of the cryoloop is made from the handle of 1 mL syringe, mechanically formed to accomodate handling and identification of the loop. Loop mounted to the stainless tube of 1 mL syringe covered by sleeved of 0.5 cryostraw. The diameter of the cover sleeved, which is just bigger than handle cryostraw allowing fixation of the sleeve on the handle cryostraw. The sleeved aimed to elude contamination that may occur in the cryo tank environment. The sleeved cryoloop then dipped in to the goblet in the liquid nitrogen.

Considering convenience of the handling procedure, total length of the sleeved cryo-loop is 12 cm. The handling part of the cryo-loop is 8.5 cm in length and it consist of fiber resistible to ultralow temperature environment of liquid nitrogen that is -196°C. Stainless mounted to the edge of the handling fiber will support confidently the loop that is made from 10.0 needle (Ethycon). The loop diameter is 700-800µm and mechanically pressed to fix to the stainless. Loop will be covered by transparent 7 cm length sleeved made from 0.5 ml cryostraw (Cassou Straw, IMV, France) allowing observation the embryos in the loop easily. Figure 1. is showing embryo that is put in the thin layer of CPA in the loop.

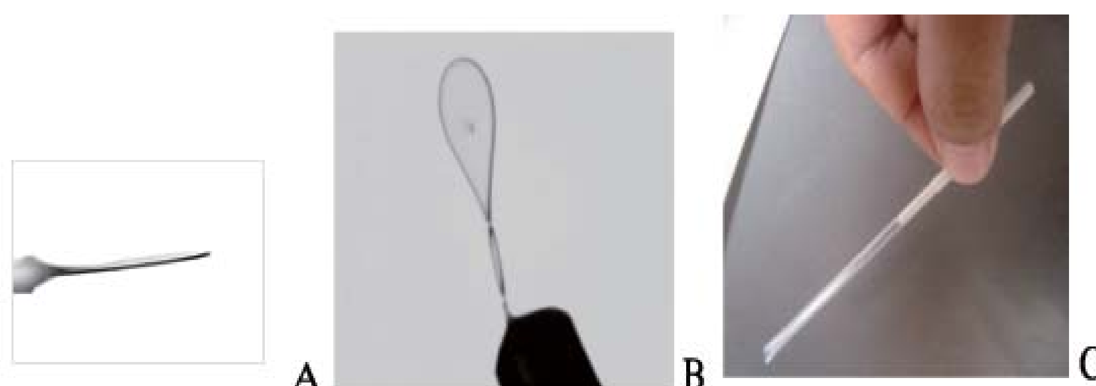

Figure 1. Loop filled by 0.1-0,2 µl CPA (A); Embryo sit on the thin CPA layer (arrow)(B) (*Ita Fauzia, 2014, Dissecting stereo Microscope, 40X, Olympus*); Gama Sleeved cryoloop (C)

Blastocyst vitrification

Procedure of vitrification was done in room temperature (25-26°C). Blastocyst were exposing in the equilibrium solution (7.5% EG (v/v); 7.5% DMSO (v/v)) for 2-3 minutes, then transferred to the vitrification solution (15% EG (v/v); 15% DMSO v/v; 10 mg/ml Ficoll; 0.65 M Sucrosa) for 30 seconds.[6] Blastocyst then inserted on to the loop of Gama Sleeved cryoloop before directly plunging into the liquid nitrogen.

Blastocyst warming

Following FET program, on the day of embryo transfer embryos were warmed rapidly by two step technique in 0.25M and 0.125M sucrosa, for 2 and 3 minutes subsequently[6]. Embryos were then cultured in the culture media untill needed. Recovered embryos were assessed for integrity of each blastomere or reexpand of blastocoele of the blastocyst. The embryos were cultured and 1 or 2 embryos with d” 25% lysis blastomere were transferred to each recipient woman. Lutheal support of 8% vaginal gel progesteron were given for 14 days until pregnancy test done. Clinical pregnancy was determined by fetal heart motion assessed by ultrasound on 5 week after embryo transfer.

RESULT AND DISCUSSION

This study describes data of the preliminary outcome of blastocyst vitrification program using 0.1µl Gama Sleeved cryoloop in our ART laboratory. Previous data reported by Desai et al, 2013 was as comparation to this present study.

A total of 97 blastocysts were vitrified and warmed from 67 patients. Ninety one blastocysts survived (93.8%) after warming. A total of 91 blastocysts were transferred into 66 patients. In one case, no blastocysts survived after vitrification and warming.

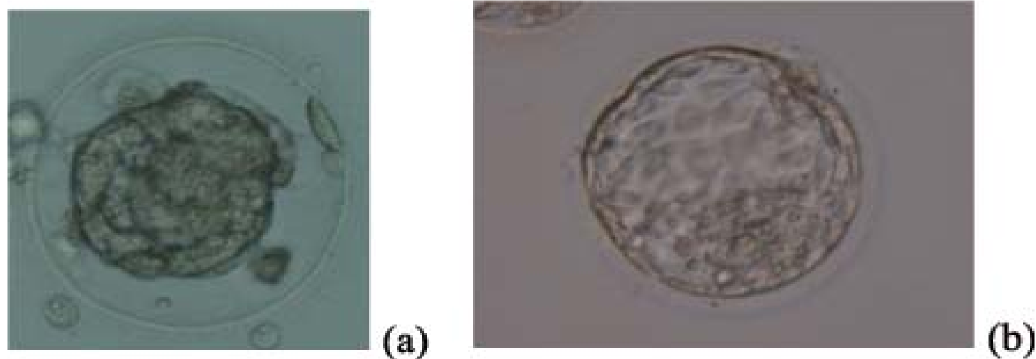

Figure 2**. Morphology of blastocyst in the vitrification solution (a) and warming solution (b) (Ita Fauzia, 2014, Inverted Microscope, 320X, Zeiss)**

Figure 2 illustrates the morphology of blastocyst in the vitrification solution. Notes the shrunken blastocyst in the vitrification solution (a) and reexpanding blastocyst (b) upon warming.

These following Table 1. is presenting the data of the outcome of blastocyst vitrification using Gama Sleeved cryoloop as well as data of blastocyst cryopreservation using loop produced by Hampton ResearchÒ.

Table 1. Vitrification outcome for blastocyst stage embryos using cryoloop (Gama Sleeved cryoloop and Hampton ResearchÒ cryoloop)

	Gama Sleeved	Hampton Research® (Desai et al, 2013)
Patients age	33 ± 2.6	34 ± 3.2
No. Vitrified warmed cycles	67	90
No. Vitrified warmed embryos	97	163
No. Intact embryos on warming (%)	91 (93.8)	148 (91)
Mean of embryos warmed	1.5 ± 0.5	1.8 ± 0.7
No. Transfer cycles	66	85
No. Embryo transferred	91	143
Mean embryo transferred	1.4 ± 0.5	1.7 ± 0.5
Implantation rate (%)	34/91 (37.4)	54/143 (38)
Clinical pregnancy (%)	29/66 (43.9)	39/85 (46)
Multiple pregnancy (%)	4/29 (13.7)	9/39 (23)
Miscarriage rate (%)	4/66 (6)	6/85 (7)
Deliveries	20	26
Infants	23	36

Of 66 transfers, 29 women conceived (43.9%). Currently, 23 baby born of 20 deliveries while another 5 ongoing pregnancies. There was 1 early pregnancy loss, 2 miscarriages at 12 weeks pregnancy, and one infant died due to a congenital anomaly. Quite similar with the data reported by Desai et al., 2013, of 85 transfer cycles, 39 women conceived (46%). Twenty six deliveries was reported with 7% misscariages.

Vitrification would be a preferred method of cryopreservation to the slow-cooling method because of the lack of ice crystal formation. In a previous data using mouse model, we reported data of vitrification method using 0.1μl Gama Sleeved cryoloop for vitrification of 8 cell to blastocyst stage embryo. The device of 0.1μl Gama Sleeved cryoloop accomodated viability of preserved embryos assessed by post warming embryogenesis and mitochondria activity.[15]

The success of vitrification procedures has recently been increased by techniques that substantially reduce the volume of vitrification solution. Among such techniques, the cryoloop must be a most refined strategy.[17,18] The loop seems accomodate very well the cooling and warming rate. Furthermore, the technique using the cryoloop is easier and simpler to be applied in our own clinic.

Refinement of culture media, based upon the physiology of the developing embryos in the human reproductive tract, it is now possible to grow viable blastocysts in vitro. This present data of human blastocyst, high survival rate may be resulted from prevented intracellular ice formation by slowly dehydrated and concentrated in the process of blastocyst vitrification[16]. This study used loop as model in the case of human setting in which the number of embryos vitrified is limited. One single human embryo of the patient will be invaluable to be sustained its vaibility through the whole procedure of vitrification and storage, thus optimally preserved the fertility of the patients.[15]

In our clinic, certain group of patients will have an opportunity to have blastocyst. Because of the resulting high implantation rates, blastocyst transfer will be a reliable approach in ART. Single blastocyst

transfer, involved in numbers of good grade blastocyst that not transferred for the fresh cycle. In this present study, we reported that vitrification using the 0.1µl Gama Sleeved cryoloop is a clinically useful method for the cryopreservation of human blastocysts. However, further study will be needed to yield an optimal outcome and thus lead the best use of valuable embryos.

ACKNOWLEDGEMENTS

The Authors thanks to Arlita Wahyu C, MD and Ngadiman for their help in the implementation of 0.1 µl Gama Sleeved Cryoloop as device for vitrification procedure at Permata Hati Infertility Clinic.

REFERENCES

1. Gardner, D.K., Schoolcraft, W.B., Wagley, L., Schlenker, T., Stevens, J., Hesla, J. 1998. A prospective randomized trial of blastocyst culture and transfer in in vitro fertilization. *Hum. Reprod.,* 13, 3434–40.
2. Frattarelli, J.L., Leondires, M.P., McKeeby, J.L., *et al.,* 2003. Blastocyst transfer decreases multiple pregnancy rates in *in vitro* fertilization cycles: a randomized controlled trial. *Fertil. Steril.,*, 79, 228-30.
3. Papanikolaou, E.G., Camus, M., Kolibianakis, E.M., Van Landuyt, L.,Van Steirteghem, A., Devroey, P. 2006. *In vitro* fertilization with single blastocyst-stage versus single cleavage-stage embryos. *N. Engl. J. Med.*, 354, 1139-46.
4. Pandian, Z., Marjoribanks, J., Ozturk, O., Serour, G., Battacharya, S. Number of embryos for transfer following in vitro fertilization or intracytoplasmic sperm injection. *Cochrane data base systematic reviews 2013*, Issue 7.
5. Thum, M.Y., Wells, V., Abdalla,H. Patients selection for blastocyst culture in IVF/ICSI treatment. J. Assist. Reprod. Genet., 27, 555-560.
6. Mukaida, T., Nakamura, S., Tomiyama, T., Wada, S., Oka, C., Kasai, M., and Takahashi, K. 2003. Vitrification of human blastocysts using cryoloops: clinical outcome of 223 cycles*. *Hum. Reprod.*, 18, (2) 384-391.
7. Kuwayama M, Vajta G, Ieda S, Kato O.2005. Comparison of open and closed methods for vitrification of human embryos and the elimination of potential contamination. *Reprod. Biomed. Online*, 11, 608–614.
8. Raju, R. 2005. Vitrification of human 8-cell embryos, a modified protocol for e better pregnancy rate. *Reprod. Biomed. Online,* 11, (4), 434-437.
9. Desai, N., Blackmon, H., Szeptycki, J., Goldfarb, J. 2007. Cryoloop vitrification of human day 3 cleavagestage embryos: post-vitrification development, pregnancy outcomes and live births. *Reprod. Biomed. Online*, 14, (2) 208-213.
10. Ishimori, H., Takahashi, Y., Kanagawa, H. 1992. Factors affecting survival of mouse blastocysts vitrifi ed by a mixture of ethylene glycol and dimethylsulfoxide. *Theriogenology,* 38, 1175-1185.
11. Mukaida, T., Wada, S., Takahasi, K., Pedro, P.B., An, T.Z., Kasai, M. 1998. Vitrification of human embryos based on the assessment of suitable condition for 8-cells mouse embryos. *Hum. Reprod.*, 13, (10) 2874-2879.
12. Kasai, M., Mukaida, T. 2004. Cryopreservation of animal and human embryos by vitrification. *Reprod. BioMed. Online* 9, 164-170.
13. Vanderzwalmen, P., Bertin, G., Debauche, C. 2003. Vitrification of human blastocysts with the Hemi-Straw carrier: application of assisted hatching after thawing. *Hum. Reprod.*, 18, 1504– 1511.
14. Kuwayama, M. 2007. Highly efficient vitrification of cryopreservation for oocyte and embryo: Cryotop method. *Theriogenology,* 67 (1) 73-80.
15. Hanoum, I.F (1Eds). Gadjah Mada (Gama) Sleeved Cryoloop as a Novel Device of Vitrification to Preserve Embryo Viability Assessed by Embryo Development and Mitochondria Activity, 2013. Aditya Media/ Program S3 FK UGM Press, Yogyakarta
16. Mukaida, T., Oka, C., Goto, T., Takahashi, K. 2006. Artificial shrinkage of blastocoeles using either a micro-needle or a laser pulse prior to the cooling steps of vitrification improves survival rate and pregnancy outcome of vitrified human blastocysts. *Hum. Reprod.,* 21, (12) 3246-3252.
17. Lane M, Schoolcraft WB, Gardner DK. Vitrification of mouse and human blastocysts using a novel cryoloop container-less technique. Fertil Steril 1999;72:1073– 8.
18. Lane M, Bavister BD, Lyons EA, Forest KT. Container-less vitrification of mammalian oocytes and embryos. Nat Biotechnol 1999;17:1234–6.