SIMPOSIO: TECNOLOGÍA DE LABORATORIO EN REPRODUCCIÓN ASISTIDA

SYMPOSIUM: LABORATORY TECHNOLOGY IN ASSISTED REPRODUCTION

RESULTADOS DEL USO DE UN SISTEMA CERRADO (S^3) EN LA VITRIFICACIÓN DE BLASTOCISTOS HUMANOS

Resumen

Este artículo describe el uso de un sistema cerrado de vitrificación que es fácil de usar y permite que los blastocitos sean vitrificados en un recipiente seguro y cerrado. Este sistema no utiliza dimetil sulfóxido (DMSO) como crío-protector y se le denomina vitrificación S3. Blastocistos humanos fueron vitrificados en los días 5 y 6 de desarrollo (día de recuperación es igual a día 0). Se conservó solo blastocistos completos de alta calidad o avanzados en el desarrollo. Se realizó un total de 139 transferencias, con una tasa de supervivencia global de 81%. La tasa de embarazos nacidos y en curso es de 58%, con una tasa de implantación de 48% para todos los pacientes en todos los grupos de edad. Los resultados globales obtenidos con este sistema cerrado son alentadores.

Palabras clave: Fecundación in vitro, vitrificación, blastocisto humano, crío-preservación

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Results following the use of a closed system (S^3) for vitrification of human blastocysts

ABSTRACT

This paper describes the use of a closed vitrification system that is easy to use and allows the blastocysts to be vitrified in a closed secure container. This system does not use dimethylsulphoxide (DMSO) as a cryoprotectant as well. This system is referred to as S3 vitrification. Human blastocysts were vitrified on Days 5 and 6 (Day of retrieval equals Day 0). Only high quality full blastocysts or greater in development were preserved. A total of 139 transfers have been performed with an overall survival rate of 81%. The delivery/ongoing pregnancy rates are 58% with a 48% implantation rate for all patients in all age groups. The overall outcomes achieved with this closed system are encouraging.

Keywords: In vitro fertilization, vitrification, human blastocysts, cryopreservation.

INTRODUCTION

With the development and refinement of extended culture media and culture systems, the consistent development of high quality blastocysts in the in vitro fertilization (IVF) laboratory has become common in human embryology today. This has greatly reduced the need to transfer more than one or two blastocysts due to their high implantation potential as compared to the transfer of cleavage stage embryos from patients with similar etiologies. Therefore, the need to cryopreserve remaining blastocysts has become very important. The ideal method should yield high survival rates, consistent embryo quality and implantation rates that are approximately equal to those obtained with fresh blastocysts. Initially, supernumerary blastocysts were cryopreserved using slow cooling methods [1, 2]. More recently, the use of vitrification to cryopreserve blastocysts has begun to increase in frequency and appears to be emerging as the methodology of choice. Vitrification is a freezing process whereby the solutions used to freeze the embryos freeze in a glass-like solid state in contrast to the crystal-like state achieved during the slow cooling process. This glass-like solid state is achieved at low temperatures by using solutions that have very high viscosity levels. In other words, the cryoprotectants are present in very high concentrations. In contrast to slow cooling, vitrification results in the elimination of ice crystals both within the cells of the embryos as well as in the surrounding solutions [3]. During the vitrification process, the blastocyst is placed into cryoprotectant solutions that maximize

cytoplasmic viscosity while inducing a strong dehydrating effect. Vitrification may prove to be superior to slow cooling because this technique avoids intracellular ice crystal formation.

Because vitrification requires the use of such viscous solutions, there exists the potential risk of exposing the blastocysts to toxic levels of highly concentrated solutions at ambient temperatures [(4)]. Therefore, most vitrification methods attempt to avoid this risk by vitrifying the embryos following very short exposure times to the cryoprotectant as well as the use of very small volumes to maximize the freezing rate [(5)]. In order to maximize cooling rates, many vitrification methodologies rely on the direct exposure of the embryo to liquid nitrogen in a very small volume of cryoprotectant. This method can pose several challenges. Namely, because of the short exposure time and use of such small volumes on minute devices, the learning curve can be rather challenging in order for the embryologist to master this technique and provide consistent outcomes from the onset. In addition, there is a risk of exposing the embryos to viral contamination as a result of direct exposure and storage in liquid nitrogen [(6)].

An alternative to using open systems to store vitrified blastocysts would be to use conventional 0.25mL sterile cryostraws. These straws are very easy to handle, load the embryos and allow the user to heat seal both ends of the straw. These straws provide a safe environment to store embryos and allow easy means of identification of the sample because of the large size.

Recently, Stachecki et al [(7)] described promising initial results using a closed DMSO-free system for vitrification of human blastocysts. This system is very easy to use because the equilibration times are longer and the time for loading the embryos and placing into liquid nitrogen is longer than open systems. We describe our experience with this system.

METHODS AND RESULTS

Only high quality full, expanded, hatching/hatched human blastocysts were selected for vitrification. These blastocysts had to possess an inner cell mass (ICM) and trophectoderm with a grade of 'B' or higher. The grading scale was a modified scoring system described elsewhere [(8)]. This method of vitrification does not require the artificial collapse of the blastocoel cavity. A series of solutions named V1, V2 and V3 were used to vitrify the blastocysts. These solutions are a phosphate buffered medium supplemented with human serum albumin (HSA) with increasing concentrations of glycerol and ethylene glycol. The blastocyst is exposed to V1 and then V2 for 5 minutes receptively. After 5 minutes in V2, the blastocyst is moved to V3 and the embryo should be loaded into the straw, sealed and placed into liquid nitrogen within 120 seconds. The configuration of the straws is shown below: Figure 1.

All embryos were stored in liquid nitrogen until thawed for replacement. Blastocysts were thawed and replaced in patients following endometrial preparation. The thaw protocol is summarized in the following diagram: Figure 2.

Figure 1. Configuration of the straws for blastocyst vitrification with V1, V2 and V3 solutions.

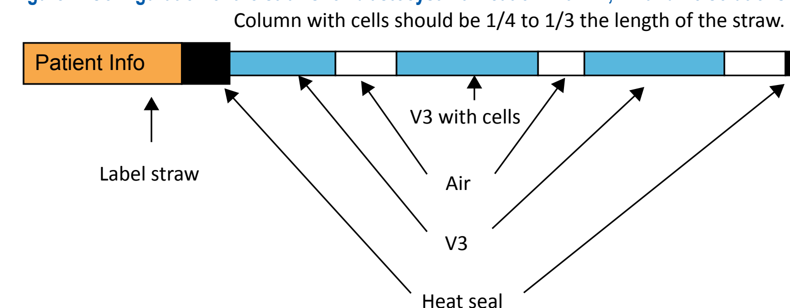
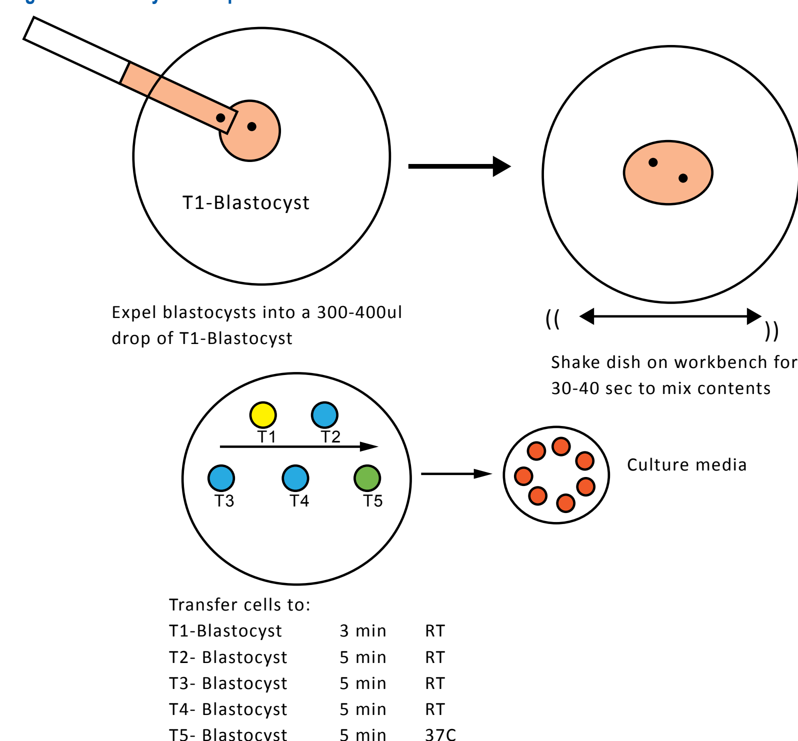


Figure 2. Blastocyst thaw protocol.



Straws were thawed in air at room temperature for 5 seconds and then submerged into a 20° C for an additional 10 seconds. Blastocysts were then expelled from the straws and the cryoprotectants were removed in a step wise procedure as depicted above. All surviving embryos were placed into equilibrated culture dishes containing global medium (IVF Online) supplements with 10% life global protein supplement (IVF Online).

Thus far, 143 patients had embryos thawed and 139 patients (97%) have had embryo transfers. The outcomes are found in table 1.
A further breakdown of outcomes by age is found in table 2.

Below is an example of what a human blastocyst looks like immediately following thawing as well as following 2 hours of culture at the time or replacement. Figure 3.

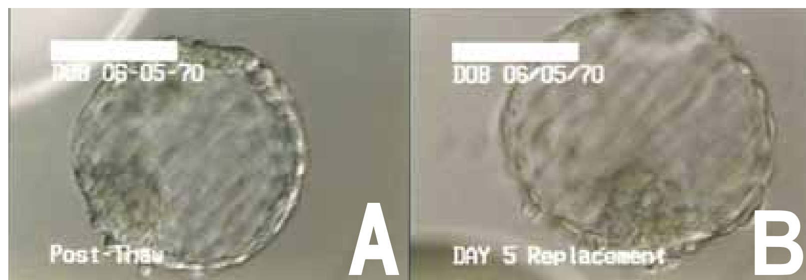
Table 1. Outcomes of embryos thawed.

Survival rate	# re expanded	+ hCG	Pregnancy rate	Implant. rate
272/334 (81%)	137/272 (50%)	105/139 (76%)	81/139 (58%)	130/272 (48%)

Table 2. Outcome of embryos thawed by patients' age.

Age	No.	Survival	Pregnancy rate
< 35	55	85	61
35-37	25	90	50
38-40	11	78	52
40-41	1	0	0
Donor oocytes	38	85	55

Figure 3. Appearance of human blastocyst: A. Immediately post thaw, B. At transfer.



CONCLUSIONS

These results demonstrate that human blastocysts can be vitrified using a DMSO-free system and stored in 0.25mL cryostraws. This technique allows for the storage of embryos in a sterile environment without direct contact with liquid nitrogen. In addition, the methods used to freeze are very simple to learn. This system allows adequate time for equilibration and loading without having to rush the protocol. The simplicity of this system should allow reproducibility across IVF centers. This system does not require that artificial collapse of the blastocoel cavity as well.

Further studies are being conducted to further optimize this system which should further improve the outcomes.

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