Comparison clinical outcomes of different post-thawed culture period of frozen-thawed single blastocyst transfer cycle

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Introduction

Over the past decade vitrification has proved to be a successful method of blastocyst cryopreservation. Many studies showed that quickly re-expansion blastocysts after warming within 2-4 hours. should be prioritized to transfer. The aim of this study was to evaluate the influence of post thawed culture period on the degree of blastocyst re-expansion and the clinical pregnancy of frozen thawed single blastocyst transfer cycle with 24 chromosome aneuploidy screening.

Material and Method

This study was a retrospective analysis of patients undergoing frozen thawed single blastocyst transfer cycle with 24 chromosome aneuploidy screening at Superior A.R.T. from November 2012 to November 2016. The inclusion criteria were:

1) Patients ≤ 38 years
2) In order to eliminate the effect of chromosome abnormalities on the clinical outcome on this study so we selected the cycle that applied 24 chromosome screening before cryopreserved by vitrification
3) Good grade blastocysts according to Gardner's criteria were selected to freeze on both day 5 and day 6
4) Only single NAD (normal as detected) embryo was transferred in frozen cycle.

A total of 586 cycles were divided into four groups according to the transfers of vitrified blastocysts (Day5 and Day6) and post-thawed culture period (2-3 hours and 4-5 hours). In particular, we compared the clinical pregnancy outcomes in transfers of frozen-thawed blastocyst and the post thawed culture period of blastocyst re-expansion in order to obtain information supporting the selection of appropriate blastocysts for vitrification and transfer. Frozen-thawed blastocysts were evaluated on the degree of re-expansion before transfer. Blastocysts with a blastocoele re-expansion of more than 50% was defined as the re-expand group. Those with a blastocoele re-expansion of less than 50% were regarded as partial re-expand group, and those not showing any blastocoele re-expansion was defined as collapse group.

Results

From 377 cycles transfer on Day 5, there is no significant difference between post-thawed culture period 2-3 hours (n=282) and 4-5 hours (n=95) on the degree of expansion (re-expansion group 83% VS 88 %, ρ-value > 0.05) and clinical pregnancy rate (51% VS 59%, ρ-value > 0.05) **(Table I)**.

Characteristic	Post thawed culture 2-3 hours	Post thawed culture 4-5 hours
Cycle (n)	282	95
re-expand rate [a]	83.0 (234/282) [b]	88.4 (84/95) [b]
Partial re-expand rate	7.8 (22/282)	4.2 (4/95)
Collapse rate	9.2 (26/282)	7.4 (7/95)
Clinical pregnancy rate [a]	51.4 (145/282) [c]	58.9 (56/95) [c]

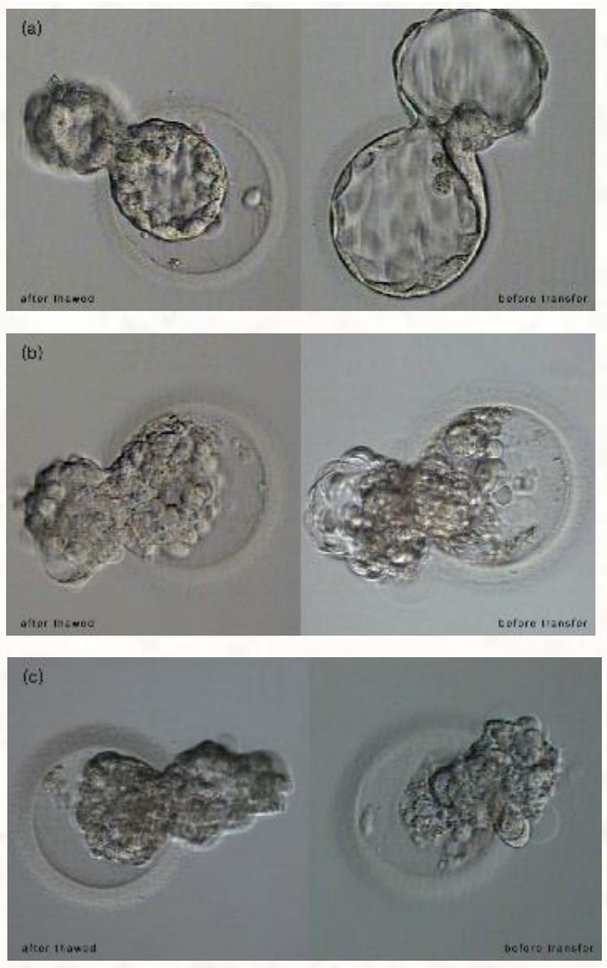
Table I. Outcomes of frozen thawed single blastocyst transfer on day 5

Results

While 209 cycles transfer on Day 6, the degree of expansion of post-thawed culture period 2-3 hours (n=152) is significantly lower than 4-5 hours (n=57) (re-expansion group 53% VS 68%, ρ-value = 0.04382) but no significantly difference of pregnancy rate between 2 groups (30% VS 21%, ρ-value > 0.05) **(Table II)**.

Characteristic	Post thawed culture 2-3 hours	Post thawed culture 4-5 hours
Cycle (n)	152	57
re-expand rate [a]	53.3 (81/152) [b]	68.4 (39/57) [b]
Partial re-expand rate	21.7 (33/152)	8.8 (5/57)
Collapse rate	25.0 (38/152)	22.8 (13/57)
Clinical pregnancy rate [a]	30.3 (46/152) [c]	21.1 (12/57) [c]

Table II. Outcomes of frozen thawed single blastocyst transfer on day 6



Frozen-thawed single blastocyst transfer cycle was carried out in hormone replacement cycles with the dose modified according to endometrial thickness and morphology (endometrial thickness reached ≥8 mm). Single blastocyst was transferred into the uterine cavity with a Soft-Trans catheter (COOK, USA) under abdominal ultra-sound guidance. Observation of a gestational sac with a beating fetal heart was defined as a clinical pregnancy.

Figure I. Photographic example of the degree of re-expansion group. (a) 100% re-expansion before transfer which defined as re-expand group. (b) < 50% blastocoele re-expansion which defined as partial re-expand group. (c) Example of no sign of blastocoele which defined as collapse group.

The degree of expansion and clinical pregnancy was compared between each group. Statistical comparisons were carried out using the chi-square test. The p-values <0.05 were considered to statistical significance.

Conclusions and discussions

Several study shown that the extended culture of post-warming blastocysts provides a chance to evaluate blastocoele re-expansion, which may take place within 1 to 2 hours after warming [1]. In addition, the results of Yin et al [2] study showed that transferring two good-morphology blastocysts frozen on day 5 that were fast re-expand within 2 hours achieved a higher pregnancy rate. Our study confirmed these findings, showing that post-thawed culture period 2-3 hours is enough to assessed re-expansion and up to 5 hours on frozen-thawed blastocyst transfer on day 5, did not effect on pregnancy rate.

In addition, no significant differences were found in the clinical outcome among the transfers of post thawed culture period 2-3 hours and 4-5 hours on day 6. Nevertheless frozen-thawed blastocyst transfer on day 6 that underwent cultured period 2-3 hours had shown the lower re-expansion blastocyst than underwent cultured period 4-5 hours. The post-thaw culture period 4-5 hours as being responsible for loss of embryo implantation and developmental potential [3]. The study was limited by the fact that post-thawed culture period 4-5 hours on day 6 had a small sample size.

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