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Blastocyst transfer after extended culture of cryopreserved cleavage embryos improves *in vitro* fertilization cycle outcomes

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ABSTRACT

Cryopreserved blastocyst embryo transfer has been reported to result in better pregnancy outcomes than those by cleavage embryo transfer. Women who had previously failed in the cleavage-stage embryo transfer, underwent extended culture of their warmed cleavage embryos to the blastocyst stage, thereby improving cryopreserved embryo transfer (CET) outcomes, although the ability of embryos to reach the extended blastocyst as well as the value of the prolonged culture was limited. This study aimed to investigate the effectiveness of blastocyst transfer by extending the culture of vitrified-warmed cleavage embryos. CET cycles were collected from January 2018 to June 2020. Pregnancy outcomes were analyzed and compared between three groups: day 2 embryo transfer using cryopreserved embryos (D2 CET), blastocyst transfer (D5 CET), and extended culture vitrified day 2 embryo transfer (D2-5 CET). A total of 52.77% of vitrified-warmed cleavage embryos developed into blastocysts in D2-5 CET group. Although D2-5 CET had a lower number of transferred embryos and grade A embryos, the pregnancy outcomes were significantly better than those in D2 CET, with respect to hCG positivity, clinical pregnancy and implantation rates (59.62% vs. 24.64%, 46.15% vs. 21.71%, 27.18% vs. 9.09%, respectively, $P < 0.05$). There were no significant different outcomes between the D2-5 CET and D5 CET groups. This study demonstrated a way of achieving better pregnancy outcomes in 8CET cycles by means of extended culture to blastocysts in patients with vitrified cleavage embryo failure.

1. Introduction

Over the past several decades, cryopreserved embryo transfer (CET) has played an important role in assisted reproductive technology (ART). Recent theoretical developments of controlled ovarian stimulation have resulted in more retrieved oocytes, thereby obtaining more embryonic development from one cycle. Cryopreservation is a good way to keep the extra number of embryos and to perform transfer cycles using cryopreserved embryos, thus increasing the cumulative pregnancy rate [25]. This approach offers a preferred method for patients with endometrial pathologies, ovarian hyperstimulation syndrome (OHSS), inadequate endometrial receptivity or excessive ovarian response [16,20]. Moreover, it allows relatively new methods to emerge, develop and be considered as pre-implantation genetic diagnosis (PGD) [16,25].

Previous published studies have compared the effectiveness of cleavage stage embryos and blastocyst stage transfer, thereby concluding that blastocyst embryos led to better pregnancy outcomes [8,16,25,26]. In theory, embryo morphology affects not only the post-warming survival rate but also the implantation rate [16]. The morphological criteria for selecting embryos on the cleavage day was less correlated with the genetic quality of the embryos because the genome was only activated when the embryos reached the blastocyst stage [13]. In practice, the blastocyst as a single embryo transfer has the potential to reduce multiple pregnancy rates [22].

Furthermore, some studies have focused on vitrification, where the stage of embryonic development takes precedence over improving pregnancy outcomes during in vitro fertilization (IVF) outcomes. Vitrification has been widely accepted as an effective cryopreservation method for human oocytes and embryos [16,21]. Blastocyst vitrification has been shown to be a better choice for embryo cryopreservation programs [2,6]. Other studies have reported that cryopreserved blastocyst transfer maintained pregnancy rates comparable to

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cleavage-stage embryo transfer [1,9]. Conversely, cleavage stage embryo transfer provided more available embryos than blastocyst transfer, resulting in greater potential for a higher cumulative live birth rate [9,11]. Only a few studies have mentioned the extended culture from cleavage stage embryos to blastocysts after warming and they suggested better outcomes from extended culture to blastocyst transfer [5,9]. Prolonged culture has been proposed as an important method because it results in more potential embryos with reduced chromosomal abnormalities [16,22].

In practice, the research question came from women who have failed in previous cleavage-stage embryo transfer, as to whether the extended culture of their warmed embryos from cleavage stage to blastocysts improved the CET. Although the number of embryos that can reach the extended blastocyst is reduced, the extended culture to blastocyst may satisfy the need for more accurate prognosis for late cleavage stage embryo transfer cycles. To fill this gap in the literature, this study aimed to investigate the effectiveness of blastocyst transfer by extending the culture of vitrified-warmed cleavage embryos.

2. Material and methods

In this retrospective study, we collected data from January 2018 to June 2020 on infertile couples who underwent vitrified-warmed embryo transfer cycles at a single-center tertiary care university hospital. This study was approved by the Ethics Committee of Hue University of Medicine and Pharmacy (approval number H2019/434). All participants provided written informed consent. The inclusion criteria included infertile couples, treated with ICSI and had CET cycles from 2018 to 2020. The exclusion criteria consisted of elderly women (over 45 years old), low responders (retrieved oocytes less than 4), oocyte donors, and endometrial thickness less than 7 mm at the time of CET.

2.1. Ovarian stimulation and oocyte retrieval

The women were treated with controlled ovarian stimulation using a gonadotropin-releasing hormone (GnRH) antagonist protocol and recombinant follicle-stimulating hormone (rFSH - follitropin alfa). On days 2–3 of the cycle, an ultrasound scan was performed to check the antral follicle count (AFC) and rule out the presence of functional cysts, and recombinant FSH (Gonal F®, Merck KGaA, Darmstadt, Germany) was administered at a starting dose of 150–225IU based on the female's age, AFC, and AMH. The GnRH antagonist, 0.25 mg/day of cetrorelix (Cetrotide®, Merck KGaA, Darmstadt, Germany), was administered from day 5 of stimulation until the day of triggering. After 35–36 h of administration of 10,000 IU human chorionic gonadotropin (hCG) (Pregnyl®, Merck Sharp & Dohme Limited, Hertfordshire, UK), oocyte retrieval was performed under transvaginal ultrasound guidance.

2.2. Intracytoplasmic sperm injection (ICSI) and embryo culture

The ICSI procedure was routinely performed in all cases. Cumulus-oocyte complexes were retrieved, washed in G-MOSP PLUS (Vitrolife®, Västra Frölunda, Sweden), and cultured in G-IVF PLUS (Vitrolife®, Västra Frölunda, Sweden) at 37 °C in an incubator, and equilibrated with 6.0% CO_2 for 2 h. The oocytes were incubated in G-IVF PLUS for 1 h before ICSI under conditions of 6.0% CO_2 and 5.0% O_2, followed by denudation using 80 IU of Hyase (Vitrolife®, Västra Frölunda, Sweden).

Sperm were prepared prior to ICSI by semen gradient concentration centrifugation using the Sil-select Plus™ density gradient system (45%–90% layers, Fertipro®, Beernem, Belgium). The sperm were then washed twice with SpermRinse (Vitrolife®, Västra Frölunda, Sweden).

After ICSI, all mature injected oocytes were individually cultured in G-TL™ (Vitrolife®, Västra Frölunda, Sweden) covered with Ovoil (Vitrolife, Västra Frölunda, Sweden) at 37 °C with 6.0% CO_2 and 5.0% O_2. The embryos were cultured on day 2 or day 5 in a benchtop incubator (IVFtech, Birkerød®, Denmark). All embryos were evaluated according to the Istanbul consensus [3]. Good- and medium-quality embryos were selected for cryopreservation by the vitrification method.

2.3. Embryo vitrification and warming

Both day 2 cleavage embryos with more than two blastomeres and less than 25% fragmentation and blastocysts on day 5 were vitrified with commercial vitrification medium (VT601, Kitazato®, Tokyo, Japan) and cryotop of Kitazato (Kitazato®, Tokyo, Japan) following the modified manufacturer's protocol. The Kitazato vitrification kit included trehalose as an extracellular cryoprotectant (ECCP) agent, which plays an important role in protecting the cellular membrane and providing more safety during the process. Hydroxypropyl cellulose (HPC) was added to reduce the risk of contamination and mechanical stress during warming. The kit comprised an equilibrated solution (ES) and vitrification solution (VS) which consisted of both dimethylsulfoxide (DMSO) and ethylene glycol (EG) as permeating cryoprotective agents. The vitrification procedure was performed at room temperature (24–26 °C) after bringing the solutions to room temperature for at least 60 min. The embryos were loaded into 150 μl of ES for 5 min with day 2 embryos and in 10–12 min with blastocysts. These embryos were then transferred to 150 μl of the first VS well for 1 min before moving to 150 μl of the second VS well in 30 s and finally loaded into a cryotop with a small volume of solution in order to obtain a high cooling rate and warming rate. The cryotop was immediately plunged in liquid nitrogen.

Subsequently, the vitrified embryos were warmed using the Kitazato Warming Kit (VT602, Kitazato®, Tokyo, Japan) according to the standard protocol. The warming kit consisted of sequential steps with ECCP in a warming solution. First, the vial containing the warming solution with ECCP was warmed to 37 °C for at least 60 min before use. The diluent (DS) and washing (WS) solutions were kept at room temperature (24–26 °C) for at least 30 min before use. The cryotop with vitrified embryos was immediately plunged into 1 ml of warming solution. After 1 min, the embryos were transferred to the first well with 150 μl of DS. The embryos were then placed in 150 μl WS in well 2 for 5 min. The last washing was performed in well 3 with 150 μl of WS in 1 min before moving to pre-equilibrated culture medium. All warmed embryos were cultured in G-TL under conditions of 6.0% CO_2 and 5.0% O_2 in an incubator at 37 °C.

The routine procedure was to warm a cryotop for each cycle of the CET. However, only half of the cleavage embryos could develop to the blastocyst stage [3]; thus, we counsel patients with more than one residual cryotop and recurrent implantation failure with two warmed cryotops in the D2-5 CET group to ensure that almost all patients had high-quality blastocyst transfer.

CET cycles were performed when the endometrium had a thickness of at least 7 mm. An artificial endometrial preparation was conducted using 4 × 2 mg tablets of Progynova (Bayer®, Leverkusen, Germany), divided into 4 mg twice daily. A 90 mg dose of Crinone Gel® 8% (Merck KGaA®, Darmstadt, Germany) was administered vaginally twice daily, starting from the night one day before plus 2 days in the D2 CET group and plus 5 days in the D5 CET group prior to transfer.

2.4. Warmed embryos culture and transfer

Vitrified cleavage embryos in D2 CET cycles and blastocysts in D5 CET cycles were cultured for 2 h prior to transfer. In the D2-5 CET group, cleavage embryos were extensively cultured to blastocysts and transferred 3 days after warming. The surviving embryo, containing more than 50% survival blastomeres and no injury to the zona pellucida, was approved immediately after warming. Embryos without damaged blastomeres were considered intact embryos [17], and only the warmed embryos, that developed into blastocysts, were transferred with the remaining embryos being discarded. The surviving embryo, containing more than 50% survival blastomeres and no injury to the zona pellucida,

was approved immediately after warming. If there were more than 2 blastocysts on day 5, the maximum number of blastocyst transfer was 2, and the surplus embryos were vitrified again. The usable embryos, considering the utilization rate of embryos, were defined as the number of embryos transferred and re-vitrified in relation to the number of vitrified embryos, thereby estimating the effect of the prolonged culture procedure due to blastocyst development potential.

In the past few years, our center has maintained two strategies; the cleavage stage and blastocyst embryo transfer. Women who failed the previous cleavage stage embryo transfer were advised to extensively undergo cultured warmed embryos from the cleavage stage to blastocysts.

The transferred embryos were placed in Embryoglue (Vitrolife, Sweden) for 15–30 min before being transferred to the uterus under ultrasound guidance using a Kitazato transfer catheter (Kitazato, Tokyo, Japan).

2.5. Clinical outcomes

Pregnancy outcomes included serum beta-hCG level, clinical pregnancy rate and implantation rate. Following this, 14 days after embryo transfer in the D2 CET group or 11 days in the D2-5 CET and D5 CET groups, beta-hCG was measured and was considered positive when it exceeded 50 mIU/mL. Clinical pregnancy was assessed by the presence of a gestational sac and fetal cardiac activity 4 weeks after embryo transfer. The implantation rate was calculated as the number of gestational sacs per transferred embryo at 6 weeks of pregnancy.

2.6. Statistical analysis

Statistical Package for Social Sciences version 20.0 (SPSS, Chicago, USA) and MedCal version 12 (MedCal Software, Ostend, Belgium) were used to analyze the data. Descriptive statistics were investigated by number and percentage for categorical data and means and standard deviations (mean ± SD) for continuous variables. Chi-square test, Student's t-test, one-way ANOVA, and Mann-Whitney test were used to compare appropriate categorical or continuous variables with the outcomes. All results were considered statistically significant at $P < 0.05$.

3. Results

The study was designed as shown in Fig. 1, involving 185 CET cycles with 525 vitrified embryos. They were separated into three groups: the D2 CET group consisted of 69 cycles with 188 embryos at the cleavage

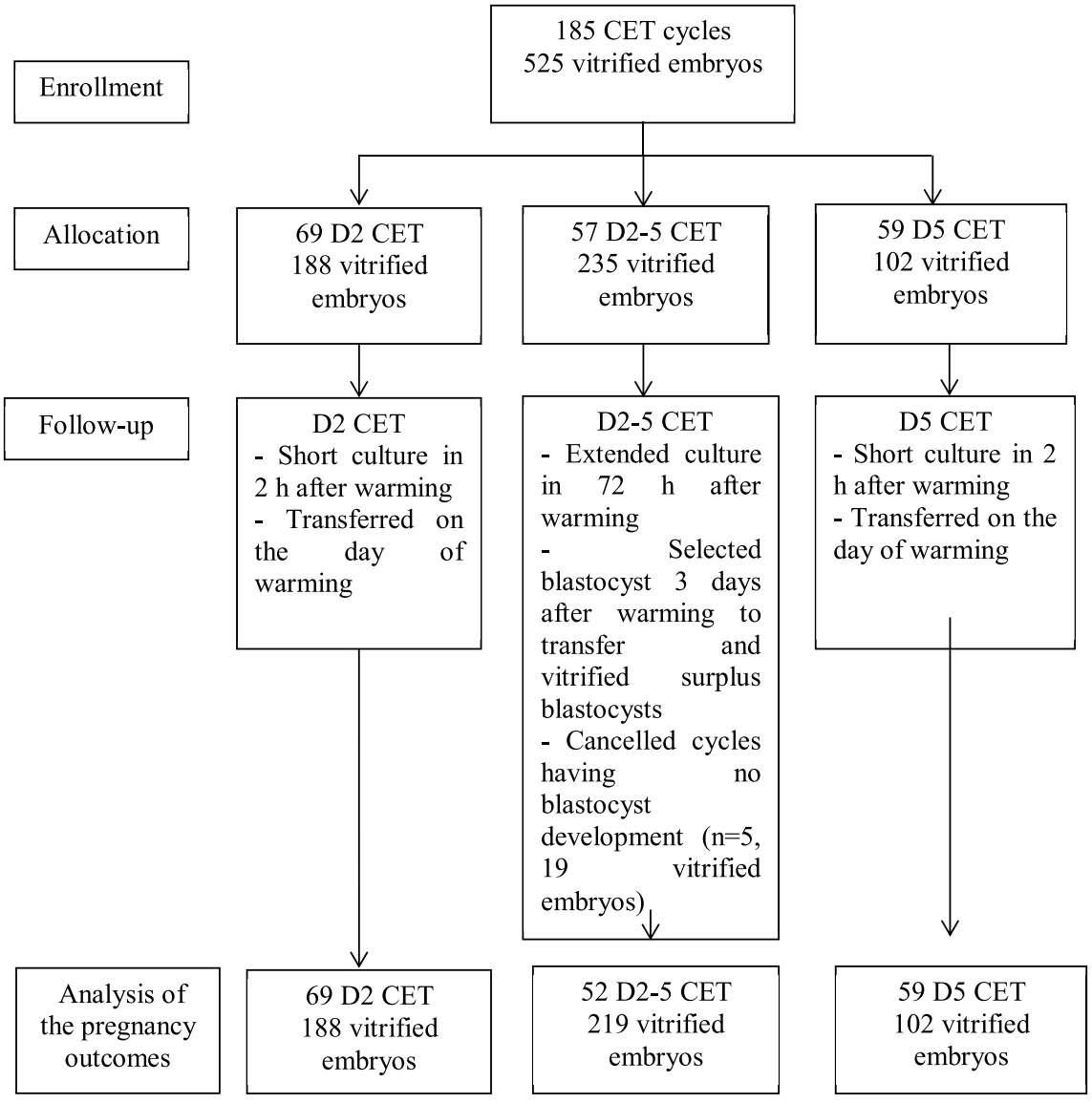

Fig. 1. Flowchart of the study sample recruitment.

stage, which were transferred to day 2 within 2 h after warming, and the D5 CET group consisted of 59 CET cycles with 102 vitrified blastocysts, which were transferred within 2 h after warming. Of the 57 cycles in the D2-5 CET group, only 52 cycles continued to metastasize, and five cases were canceled because they did not develop into blastocysts.

The baseline characteristics of the participants demonstrated that there were no significant differences between the three groups with respect to women's age at the ICSI cycle treatment, duration and type of infertility, geographic location, and endometrial thickness. However, the mean age of women in the D2-5 CET group was significantly higher than that in the D5 CET group at the time of transfer (Table 1).

As shown in Table 2, the mean of vitrified embryos in the D2-5 CET group (4.12 ± 1.39) was statistically higher than that in D2 CET (2.72 ± 0.62) and D5 CET groups (1.73 ± 0.52). This group required warming more vitrified embryos per embryo transfer cycle than the D2-and D5-CET groups. Vitrification had excellent intact embryo rates in the three groups (99.24% in general). Much attention has been paid to decrease the number of grade A embryos transferred after extended culture from the day 2 vitrified embryos of the D2-5 CET group. The failure of the blastocyst development process in extended vitrified day 2 embryos diminished the number of transferred embryos. It was shown that the percentage of usable embryos in the D2-5 CET group raised 52.77% (124/235), of which there were 103 transferred blastocysts and 21 re-vitrified blastocysts.

Although the mean number of transferred embryos and grade A embryos per cycle in D2-5 CET was significantly lower than that in D2 CET (1.81 ± 0.83 vs. 2.71 ± 0.62, 0.77 ± 0.77 vs. 1.8 ± 1.01, respectively), Table 3 showed that extended vitrified cleavage embryo transfer (D2-5 CET) had significantly better pregnancy outcomes than vitrified cleavage embryo transfer (D2 CET) with respect to hCG positive rate, clinical pregnancy rate and implantation rate (59.62% vs. 24.64%, 46.15% vs. 21.71%, 27.18% vs. 9.09%, respectively with $P < 0.05$). Interestingly, similar outcomes were recorded in D2-5 CET and D5 CET while D2-5 CET had fewer grade A embryos transferred per cycle than D5 CET..

Table 1
Basic characteristics of the study population.

Characteristics	D2 CET 69 cycles 188 embryos	D2-5 CET 57 cycles 235 embryos	D5 CET 59 cycles 102 embryos	Total 185 cycles 525 embryos	P value
Women's age at ICSI cycles	33.14 ± 5.06	32.39 ± 4.56	31.69 ± 4.56	32.42 ± 4.8	0.05
Women's age at CET	33.29 ± 5.09	34.11 ± 4.52[a]	31.71 ± 4.56[a]	33.03 ± 4.86	0.022
Infertility duration (years)	5.43 ± 3.25	4.63 ± 2.79	4.85 ± 2.72		
<3	13 (18.8)	10 (19.2)	7 (11.9)	30 (16.7)	> 0.05
≥3	56 (81.2)	42 (80.8)	52 (88.1)	150 (83.3)	
Infertility type					
Primary	49 (66.7)	33 (63.5)	47 (79.7)	129 (71.7)	> 0.05
Secondary	20 (33.3)	19 (36.5)	12 (20.3)	51 (28.3)	
Geography					
Urban	43 (62.3)	29 (55.7)	27 (45.8)	99 (55)	> 0.05
Rural	26 (37.7)	23 (44.3)	32 (54,2)	81 (45)	
Endometrial thickness (mm)	9.54 ± 2.05	9.28 ± 1,22	9.46 ± 1.65	9.46 ± 1.66	> 0.05

CET: Cryopreserved embryo transfer; ICSI: intracytoplasmic sperm injection. All continuous variables are expressed as mean ± standard deviation (mean ± SD).

[a] P value < 0.05: D2-5 CET vs. D5 CET (P = 0.005).

4. Discussion

The present study aimed to investigate the effectiveness of blastocyst transfer via extended culture of their warmed embryos from the cleavage stage, due to the need for women who failed in previous cleavage stage embryo transfer and wished to have better outcomes.Our data showed dramatically higher pregnancy outcomes in extended culture and blastocyst transfer than in vitrified cleavage stage embryo transfer.

In recent years, blastocyst transfer has been widely adopted as the preferred option in IVF cycles. Benefiting from many achievements in ART, including commercialization of good quality culture media, optimization of clean room conditions, and incubator innovation, blastocyst stage development has achieved the great results, and blastocyst transfer has shown a high implantation rate both in fresh and in CET cycles [9,11,12,15,18]. However, some previous studies have reported results contrary to our findings. Singh and Singh (2013) reported that pregnancy outcomes were not different and that the cleavage stage (day 3) transfer could be better and more cost-effective than blastocyst transfer [24]. In recent literature, embryo quality has been found to be correlated with implantation rate [13]. Nevertheless, the cleavage stage had disadvantages in embryo selection. Using a combination of FISH and CGH techniques to detect chromosomal abnormalities, Dekel-Naftali et al. (2013) indicated a higher level of abnormalities in cleavage embryos [7]. Regardless of the best morphology on day 3, chromosomal abnormalities continued to appear, while poor-quality embryos developed to the blastocyst stage with a high level of abnormalities [10]. Embryo selection for transfer at the cleavage stage seems to be problematic because the selection of cleavage embryos has a weak relationship with the embryo genome [8]. On the other hand, embryo selection at the blastocyst stage may lead to better embryo transfer, resulting in a higher pregnancy rate. Previous studies have confirmed that the aneuploidy of high-quality embryos on day 3 was 59%, while abnormalities in a good blastocyst accounted for only 35%. Aneuploidy represented in the blastocyst stage at a low level could improve the pregnancy outcomes of blastocyst transfer compared to cleavage embryo transfer [19]. With vitrified cleavage stage embryos, the extended culture of embryos from the cleavage stage to blastocyst which enhanced embryo selection, resulted in higher pregnancy outcomes similar to those of vitrified blastocysts.

Logically, blastocyst transfer would increase endometrial synchronicity, as well as increase the ability to select the best blastocysts, which is why a higher live birth rate was scored. In addition, the selection of high-quality blastocysts has become a strategy for single blastocyst transfer to reduce the risk of multiple pregnancies associated with the risk of obstetric and neonatal complications [1,8,21]. Furthermore, blastocyst culture provided the opportunity to undergo PGD-assisted single-embryo transfer. Compared to fresh transfer, cryopreserved blastocyst transfer could also prevent the negative effects of endocrine fluctuation, resulting from controlled ovarian hyperstimulation [23]. Vitrification appeared to be the best method of embryo cryopreservation at all stages with a high post-warming embryo survival rate, more potential to replace fresh transfer in cases of OHSS, and poor endometrium with surplus embryos [28]. Therefore, blastocyst transfer after extended culture of vitrified cleavage stage embryos is an appropriate strategy in IVF cycles, especially in cases where the previous embryo transfer failed.

In our study, the success of the extended embryo culture from day 2 vitrified-warmed embryos, as shown by the 52.77% of usable embryos and 5 cycles, did not always succeed in transfering because the development of cleavage embryos was prevented and blastocyst formation was not observed. A major disadvantage of blastocyst transfer is cycle cancelation. Kasraie reported that cycle cancelation was higher in blastocyst cycles than in cleavage stage cycles (8.9 vs. 2.8%) [14]. Our data found no cycles canceled when transferring vitrified-cleavage embryos (D2 CET) and blastocysts without prolonged culture (D5 CET).

Table 2
The quality of cryopreserved embryos of the three groups.

Characteristics	D2 CET 69 cycles		D2-5 CET 57 cycles		D5 CET 59 cycles		Total 185 cycles	
	n (%)	(mean ± SD)	n (%)	(mean ± SD)	n (%)	(mean ± SD)	n (%)	(mean ± SD)
Nr of vitrified embryos	188 (100)	2.72 ± 0.62[a,b]	235 (100)	4.12 ± 1.39[a,c]	102 (100)	1.73 ± 0.52[b,c]	525 (100)	2.81 ± 1.32
Nr of warmed cryotops	69 (100)	1[a]	86 (100)	1.51 ± 0.5[a,c]	59 (100)	1[c]	214 (100)	1.16 ± 0.36
Nr of vitrified grade A embryos	124 (65.96)	1.8 ± 1.01[a,b]	151 (64.26)	2.65 ± 1.34[a,c]	68 (66.67)	1.15 ± 0.58[b,c]	338 (64.38)	1.85 ± 1.18
Nr of embryos intact after warming	187 (99.47)	2.71 ± 0.62[a,b]	233 (99.15)	4.09 ± 1.39[a,c]	101 (99.02)	1.72 ± 0.53[b,c]	521 (99.24)	2.81 ± 1.31
Usable embryos	187 (99.47)	2.71 ± 0.62[a,b]	124 (52.77)	2.18 ± 1.18[a,c]	101 (99.02)	1.72 ± 0.53[b,c]	412 (78.48)	2.23 ± 0.91
Transferred embryos	187 (99.47)	2.71 ± 0.62[a,b]	103 (43.83)	1.81 ± 0.83[a]	101 (99.02)	1.73 ± 0.52[b]	392 (77.27)	2.12 ± 0.81
Transferred grade A embryos	124 (65.96)	1.8 ± 1.01[a,b]	44 (18.72)	0.77 ± 0.77[a,c]	67 (65.69)	1.14 ± 0.57[b,c]	235 (46.44)	1.27 ± 0.92

SD: standard deviation; CET: Cryopreserved embryo transfer; Nr: number.
[a] P value < 0.05: D2 CET vs. D2-5 CET.
[b] P value < 0.05: D2 CET vs. D5 CET.
[c] P value < 0.05: D2-5 CET vs. D5 CET.

Table 3
Pregnancy outcomes of cryopreserved embryo transfer cycles.

Characteristics	D2 CET 69 cycles	D2-5 CET (*) 52 cycles	D5 CET 59 cycles	P value
hCG positive rate (%)	24.64[a,b] (17/69)	59.62[a] (31/52)	50.8[b] (30/59)	< 0.05
Clinical pregnancy rate (%)	21.71[a,b] (15/69)	46.15[a] (24/52)	45.8[b] (27/59)	< 0.05
Implantation rate (%)	9.09[a,b] (17/187)	27.18[a] (28/103)	32.67[b] (33/101)	< 0.05

CET: Cryopreverved embryos transfer; (*): D2-5 CET group excluded 5 cases having no blastocyst transferred.
[c] P value < 0.05: D2-5 CET vs. D5 CET.
[a] P value < 0.05: D2 CET vs. D2-5 CET.
[b] P value < 0.05: D2 CET vs. D5 CET.

According to reports, not only was the quality of embryos on day 3 good, but the poor quality may also develop into blastocysts, resulting in a successful clinical pregnancy [4,13,27]. Sallem et al. (2018) also reported that low-scoring embryos from day 2 were able to develop into blastocysts and resulted in healthy babies [21]. These findings suggest that embryos can be cultured extensively without morphological or embryological criteria [9].

Our results revealed that the extended culture cleavage embryo transfer consumed more embryos and less usable embryos than cleavage embryo transfer. Only half of the cleavage stage can develop into blastocyst stage embryos [8,27]. In our study, we had to warm more embryos in the D2-5 CET group to ensure that almost all patients had high-quality blastocysts for transfer. Although the usable embryo rate in the D2-5 CET group and the mean value of transferred embryos were much lower than that of the D2 CET group, D2-5 CET had surplus re-vitrified embryos after transfer. These re-vitrified embryos may be the more opportunity for patients who need repeated CET because of an unsuccessful pregnancy from embryos twice vitrified-warmed [5,25]. In spite of less grade A embryos transferred, pregnancy results of D2-5 CET were much better than those of D2 CET and had similar outcomes to D5 CET. As a result, lesser blastocyst embryo transfer can result in higher pregnancy outcomes, and patients are more likely to be transferred by these extra blastocysts in future cycles. In any case, patients should be counseled about the risk of blastocyst cycle cancelation before treatment.

A limitation of our study was that we did not have criteria to determine the number of vitrified cleavage embryos to be warmed. Since the vitrification procedure is a very good cryopreservation method with an excellent rate of intact embryos (over 99%), more studies should be performed to determine the optimal number of embryos selected for extensive culture. In addition, more embryos, laboratory staff, equipment and much cost were required to extend the culture to the blastocyst stage. A cost-effectiveness analysis should be performed to confirm the economic impact of this strategy. Based on the results of this study, the extended culture of vitrified cleavage embryos should be specified for specific conditions. Patients who have failed previous vitrified cleavage embryo transfer should be counseled to transfer with or without extended embryo culture up to the blastocyst stage despite improving the chance of successful pregnancy.

In conclusion, our study confirmed a better pregnancy outcome in CET cycles by extending culture to blastocysts, and vitrified blastocyst transfer cycles are preferred in patients with failed cleavage embryos. Further studies should be performed to determine the optimal cleavage embryos to be selected in order to ensure the formation of good-quality blastocysts and to balance the cost-effectiveness of this approach.

Ethics approval and consent to participate

This study was approved by the Hue University of Medicine and Pharmacy Ethics Committee (approval number H2019/434).

Availability of data and material

The datasets used and/or analyzed during the current study are available from the corresponding author upon reasonable request.

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CRediT authorship contribution statement

Minh Tam Le: Supervision, Conceptualization, Methodology, Reviewing and Editing: Thai Thanh Thi Nguyen, Trung Van Nguyen and Hong Nhan Thi Nguyen: Conceptualization, Data collection, Methodology, Formal analysis, Writing, Original draft preparation; Quoc Huy Vu Nguyen: Conceptualization, reviewing and editing. All authors contributed to the interpretation of the data and have approved the final manuscript.

Declaration of competing interest

The authors declare no conflict of interest. The authors alone are responsible for the content and writing of this article.

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