

# Outcomes of embryo vitrification at different developmental stages

## Evaluation of 2412 warming cycles

Lizhen Xu, MD<sup>a</sup>, Shanshan Gao, MD<sup>b</sup>, Jingjing Jiang, MD<sup>b</sup>, Mei Sun, MD<sup>b</sup>, Yan Sheng, MD<sup>b</sup>, Rong Tang, MD<sup>b,\*</sup> 

### Abstract

**Introduction:** Advances in cell culture media have led to a shift from cleavage stage embryo transfer to blastocyst stage transfer. Extended embryo culture to the blastocyst stage provides some theoretical advantages and disadvantages. There has been controversy. This study is sought to evaluate the clinical outcomes of vitrified-warmed cleavage-stage and blastocyst-stage embryo transfers in patients undergoing Artificial Reproductive Technique treatments.

**Material and method:** The study was performed on 2740 women undergoing frozen embryo thawing transfer. Patients' basic situation, status of frozen embryo transfer cycle, clinical pregnancy rate, early abortion rate, sex ratio of birth, and birth weight were retrospectively analyzed. We compared the main clinical results of recovery of frozen embryo on the cleavage stage and blastocyst stage. Furthermore, we evaluated the clinical outcomes of blastocyst cryopreservation on Day 5, 6, or 7 after oocyte retrieval according to the day of blastocyst expansion were evaluated.

**Results:** The implantation ratio of cleavage stage embryos was 21.62% compared with 43.52% on D5 ( $P < .05$ ). The D5, D6, and D7 implanting rates were statistically different. The pregnancy rates were 57.56%, 51.76%, and 35.95% versus 37.79%, respectively for embryos cryopreserved on D5, D6, D7, and D3. The ectopic pregnancy rate and early abortion rate were statistically different between D5 and D3. The sex ratio, the birth weight, and birth defect were not statistically different among the four groups.

**Conclusions:** Blastocyst transfer achieved a higher implantation rate than vitrified cleavage stage embryo and decreased ectopic pregnancy rate. With increased incubation days before expansion blastocyst formed, the implantation rate is reduced and the early abortion rate increases.

**Abbreviations:** ART = Artificial Reproductive Technique, ET = Embryo Transfer, MC = menstrual cycle, SART = the Society for Assisted Reproductive Technology.

**Keywords:** blastocyst, cleavage stage, clinical outcome, vitrified-warmed

### 1. Introduction

In recent years, in most reproductive centers in China, embryo transfer is preferred over D3 embryos transfer, and the remaining embryos at the cleavage stage are cryopreserved or

cultured into blastocysts. Currently, with the rapid development of vitrification technology and blastocyst culture programs, blastocyst transplantation has become more and more common and worldwide in the clinical practice of Artificial Reproductive

Editor: Roxana Covali.

This program was supported by the Projects of medical and health technology development program in Shandong Province (No. 2016WS0368) and the Projects of Reproductive Research Center of Shandong University (No. 2014GSF118148).

This study was approved by the Ethics Committee of the provincial hospital, and all participants signed a consent form prior to the study.

Written informed consent was obtained from the patient/participant (which will be removed as appropriate) with their personal details and accompanying images in this manuscript. The consent form is kept by the authors/by the authors' institution/in the patients' clinical notes (removed as appropriate) and is available for review by the Editor-in-Chief.

The data and materials used in this study can be provided according to the reasonable requirements of the corresponding author once the paper has been published.

The authors have no conflicts of interest to disclose.

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

<sup>a</sup> Department of Reproductive Medicine, Renji Hospital, School of Medicine, Shanghai Jiaotong University, Shanghai 200001, China, <sup>b</sup> Center for Reproductive Medicine, Provincial Hospital Affiliated to Shandong University, Jinan 250021, China.

\* Correspondence: Rong Tang, Center for Reproductive Medicine, Provincial Hospital Affiliated to Shandong University, Jinan 250021, China (e-mail: tangtangtang\_008@sohu.com).

Copyright © 2022 the Author(s). Published by Wolters Kluwer Health, Inc.

This is an open access article distributed under the terms of the Creative Commons Attribution-Non Commercial License 4.0 (CCBY-NC), where it is permissible to download, share, remix, transform, and buildup the work provided it is properly cited. The work cannot be used commercially without permission from the journal.

How to cite this article: Xu L, Gao S, Jiang J, Sun M, Sheng Y, Tang R. Outcomes of embryo vitrification at different developmental stages: evaluation of 2412 warming cycles. *Medicine* 2022;101:19(e29233).

Received: 28 June 2020 / Accepted: 16 March 2022

<http://dx.doi.org/10.1097/MD.00000000000029233>

Technique (ART).<sup>[1,2]</sup> However, the optimal time for embryo transfer remains controversial.

Many studies by Cochrane Review<sup>[3]</sup> have demonstrated the theoretical advantages of blastocyst transplantation: better correlation between morphology and aneuploidy, better synchronization with endometrium, and increased implantation potential. On the other hand, there are some defects in blastocyst transplantation, such as the lack of co-culture with endometrial cells for one day (embryo enters the uterine cavity on the 4th day), which increases the possibility that some embryos will not develop into blastocysts in vitro, resulting in the abolition of embryo transfer; the decrease of embryo freezing rate is related to the decrease of co-culture rate of endometrial cells. There are technical differences in the expansion embryo during freezing/thawing. Therefore, the contradictory results recently reported by Cochrane meta-analysis suggest that there is no evidence of differences in pregnancy outcomes between 2–3rd and 5–6th after embryo transfer.<sup>[4]</sup>

A recent Cochrane meta-analysis found no significant differences in live birth or pregnancy outcomes between 2–3rd and 5–6th embryo transfer.<sup>[5]</sup> Moreover, blastocyst transfer increased the failure rate of any embryos transferred in one cycle, reducing the embryo freezing rate. In addition to the success achieved through fresh embryo transfer, the subsequent embryo cryopreservation cycle of ART provides further possibilities for success.<sup>[6,7]</sup> Therefore, the cumulative pregnancy rate after fresh and additional vitrification of each oocyte retrieval cycle may be considered more accurate clinical outcomes than simply the pregnancy rate per cycle of embryo transfer.

Our study retrospectively analyzed clinical pregnancy outcomes, follow-up analysis, and perinatal outcomes of Chinese women with transfer of vitrified-warmed D3 embryos, D5 blastocysts, D6 blastocysts, and D7 blastocysts after ART treatments in China.

## 2. Materials and methods

From January to December 2011, a study of 2740 women who underwent frozen embryo thawing transfer came to the clinic in Reproductive Hospital Affiliated to Shandong University. Since D3, D5, D6, or D7 embryos were thawed in the same cycle, we eliminated 157 cycles. Another 171 cycles were eliminated for embryo death. A total of 2412 Frozen Embryo Transfer cycles, of which 172 cycles were frozen embryo transfer on D3, 721 cycles were transferred on D5, 1366 cycles were transferred on D6, and 153 cycles were transferred on D7. Due to the small number of high-quality D3 embryos, the frozen embryo transfer period of the cleavage stage is short, which reduces the chance of blastocyst embryos developing into blastocysts. The patients' basic situation, frozen embryo transfer cycle status, clinical pregnancy rate, early abortion rate, sex ratio at birth, and birth weight were retrospectively analyzed.

### 2.1. Natural cycle

In patients who used this regime with regular menstrual cycle (MC), the follicular growth was monitored by measuring the levels of hormones in the serum and performing ultrasound from cycle day 10. When the diameter of the dominant follicle was >16 mm and the endometrial thickness was >8 mm, with estradiol (E2) >150 ng/L and progesterone (P) <1.0 µg/L, 5000 IU hCG (Lizhu Pharmaceutical Trading Co., Zhuhai, China) was

administered to trigger ovulation, and embryos transfer was performed 3 or 5 days later.

### 2.2. Ovulation induction endometrial preparation

Patients were administered Letrozole (Jiangsu Hengrui Medicine Co., Ltd, Lianyungang, China) 2.5 to 5.0 mg/d or Human Menopausal Gonadotropin 75 to 150 IU injection on day 3 of the MC, with continuous medication for 3 days or 5 days. In patients with irregular MC (≥34 days), follicular development was monitored using ultrasonography starting on day 10 of the MC. Serum follicle-stimulating hormone, LH, E2, and P concentrations were measured on the same day as the ultrasound examination. If the diameter of dominant follicles were <16 mm, then injections of h MG (Lizhu Pharmaceutical Trading Co., Zhuhai, China) 150 IU were given continually. When the diameter of dominant follicles were ≥16 mm, endometrial thickness ≥8 mm, and E2 ≥150 ng/L, the level of LH determines hCG injection time. If LH was <20 IU/L, 5000 IU hCG was administered to trigger ovulation, and embryos transfer was performed 3 or 5 days later.

### 2.3. Hormone Replacement Therapy endometrial preparation

Hormone Replacement Therapy focused on recurrent implantation failure, MC <23 days, prolonged menstruation, ovulation bleeding, or patients with thin endometrium in NC or ovulation cycles. Hormone Replacement Therapy referred to the oral administration of ethinyl E225 mg tid (Xinyi Pharmaceutical Co., Shanghai, China) from day 3 of MC onward with continuous medication for 14 days. Endometrial development was monitored using ultrasonography starting on day 17 of MC. Serum follicle-stimulating hormone, LH, E2, and P concentrations were measured on the same day as the ultrasound examination. If the endometrial thickness was >8 mm, P should be given for endometrial transformation, embryo transfer was scheduled 3 to 5 days later.

### 2.4. Protocol for vitrification and warming

Blastocysts were vitrified by traction cutting and cut with 0.25 ml plastic sterile straw (Bicef). Blastocysts were equilibrated in 7.5% ethylene glycol and 7.5% dimethyl sulfoxide for 20 to 25 minutes, exposed to phosphate buffer saline in 15% ethylene glycol and 15% dimethyl sulfoxide, and added 0.5 M sucrose within 1 minute. Then they were immediately put into liquid nitrogen.

Before recovery, we prepared 1 mL solution containing 0.33 M sucrose, 0.2 M sucrose, and 0 M sucrose for four three-well culture plates and kept them at 37°C for at least 30 minutes. We removed the recovery blastocyst and the carrier shell and placed the carrier terminal into the prepared 0.33 M sucrose within 2 minutes, then we transferred embryos into 0.2 M sucrose within 3 minutes, and finally placed the blastocyst into the BS within 5 minutes. Recovery of good blastocysts was achieved using laser-assisted hatching. We punched away from the transparent cell mass because properly sized cell-sized holes are best. Then, we put the blastocysts into blastocyst medium of G2 (Vitrolife, Sweden) at 37°C and 6% CO<sub>2</sub> and cultivated it for 5 hours in saturated humidity to prepare for transplantation.

**Table 1**  
**SART scoring system.**

Rating scale	Characteristics of embryo	
	Fragment ratio	Homogeneity
Good	<10%	Homogenization
Middle	11%–25%	Moderate
Poor	>25%	Highly uneven

SART = the Society for Assisted Reproductive Technology.

**2.5. Luteal support and pregnancy confirmation**

Progesterone was administered by injections after Embryo Transfer (ET) (40–80mg) or orally (20–40mg) daily. In all groups, serum β-hCG tests were performed 2 weeks after ET. Transvaginal ultrasound was scheduled to confirm a clinical pregnancy after 3 weeks.

**2.6. SART embryo scoring system**

The SART scoring system is an evaluation criterion proposed by the Society for Assisted Reproductive Technology (SART) and widely used in its member institutions. The SART score classifies embryos into good, medium, and poor, with detailed scoring rules as shown in Table 1.

**2.7. Statistical analysis**

Statistical analysis was performed using SPSS (Statistical Package for the Social Science; SPSS Inc., Chicago, IL). The results are expressed as a percentage. Ordinary values such as rate comparisons were analyzed by the χ<sup>2</sup>-test. Regression analysis was implemented to investigate the correlation between multiple variables and implantation rate. A value of *P* < .05 was statistically significant.

**3. Results**

After excluding 157 cases who received embryos transfer from D5, D6, or D7 in the same cycles, a total of 2583 warming cycles were included; 2412 cryotransfers were performed with an ET cancellation rate of 6.62%. Patients undergoing blastocyst-stage ET were younger than those undergoing cleavage-stage embryo transfers (*P* < .05). The proportion of ETs was lower when embryos were vitrified on day 3 (*P* < .05). (Table 2)

The clinical pregnancy rate was 51.49%, and the miscarriage rate was 20.61%. A total of 421 cleavage-embryos were transferred (mean: 2.457±0.62) in 172 cycles, of which 91 embryos were successfully implanted (overall implantation 21.62%). A total of 3603 blastocysts were transferred (mean:

1.61±0.49) in 2240 cycles, of which 1568 blastocysts were implanted (overall implantation 43.52%). Clinical pregnancy rates, ectopic pregnancy, and miscarriage rates differed among vitrified embryos on D3, D5, D6, and D7 (Table 3).

Among 1242 pregnancies, there were 970 live born (78.10%), of which 576 were singletons (59.39%), 373 were twins (38.45%), and 21 were triples (2.16%). Mean gestational age, birth weight, sex ratio, and birth defects for different groups (Table 4).

**4. Discussion**

Traditionally, cleavage-stage embryos were transferred or cryopreserved on the 3rd day, but in the past decades, there had been a tendency to transfer blastocyst embryos.<sup>[8]</sup> With the development of efficient culture systems, obtaining blastocysts in vitro has become increasingly reliable. Blastocysts are preimplantation embryos that have successfully passed the critical step of genomic activation and have a high developmental potential.<sup>[9]</sup> In addition, blastocyst transferring is more physiologically appropriate because it is closer to the time of natural implantation and may improve synchronization of the endometrium and embryo development.<sup>[10,11]</sup> In our study, the implantation and clinical pregnancy rates were statistically lower in the D3 group than those in the blastocyst groups (21.62% vs. 43.52%, 37.79% vs. 52.54%).

In this study, vitrification technology was used to freeze embryos and blastocysts. Due to the higher survival rate, the blastocyst transfer on day 5 had statistically lower cancellation rate. The presence of the mitotic apparatus in cleavage embryos might be disturbed during vitrification procedures. Another explanation might be that D3 embryos vitrification might carry out when a patient has achieved relatively few high morphologic quality embryos, which was considered as another explanation and also explained why patients undergoing early cleavage-stage vitrification were older than those undergoing D5 blastocyst vitrification.<sup>[12,13]</sup>

Although blastocyst transfer has various advantages, due to suboptimal culture conditions, embryo development in vitro may be slightly delayed in general. Mature blastocysts typically develop in vitro until day 5, whereas the mean age of in vivo matured blastocysts collected by uterine lavage was reported to be 4.5 days after ovulation.<sup>[14]</sup> Both differentially developed blastocysts (day 5 vs. day 6 vs. day 7) and year-round cleavage embryos (day 3) were involved in the present study. The proportion of blastocyst vitrifying-warming cycles on day 6 was higher than that on day 5 and day 7. Evidence from the timing of pantopod appearance, histological features, and steroid receptor down-regulation indicate that the stimulated receptor window may move slightly earlier than the natural

**Table 2**  
**The frozen blastocyst transplant cycle.**

	D3 cleavage stage embryo	D5 blastocyst	D6 blastocyst	D7 blastocyst
Thaw cycles	187	736	1477	183
Give up cycles (rate)	15 (8.02%)a	15 (2.04%)b	111 (7.52%)a	30 (16.39%)c
Transplantation cycles	172	721	1366	153
Average age (y)	33.0±4.26a	30.72±4.41b	31.40±4.63c	32.88±5.02a
Average number of embryos	2.45±0.62a	1.58±0.49b	1.63±0.49c	1.49±0.51d
Lining thickness (cm)	1.01±0.18	1.01±0.16	1.02±0.16	1.02±0.15

a.b.c.d.: Statistically different between different peer letters (*P* < .05).

**Table 3**

**Different culture times of frozen blastocysts compared to clinical outcomes after recovery.**

Item	D3 cleavage-stage embryo	D5 blastocyst	D6 blastocyst	D7 blastocyst
Cycles	172	721	1366	153
Embryo transfer number	421	1142	2233	228
7-Week clinical pregnancy	91	569	937	62
Implantation rate	21.62%a	49.82%b	41.96%c	27.19%a
Clinical pregnancy (rate)	65 (37.79%)a	415 (57.56%)b	707 (51.76%)b	55 (35.95%)a
Ectopic pregnancy (rate)	3 (4.62%)ab	6 (1.45%)a	7 (0.99%)ac	1 (1.82%)a
Early abortion cycles (rate)	15 (23.08%)a	64 (15.42%)ab	158 (22.35%)ac	19 (34.55%)ad

a.b.c.: Statistically different between different letters in the peer ( $P < .05$ ).

cycles,<sup>[15]</sup> increasing the likelihood that later-developed blastocysts will miss the implantation window. Nevertheless, whether the delayed development in vitro would influence the clinical and perinatal results in warming cycles was controversial.<sup>[16]</sup> The results of this study showed that the clinical pregnancy rate and lower miscarriage rate of the three groups of blastocysts with different developmental levels on day 5 after warming have increased statistically. Also, the implantation rate of D7 blastocyst cryopreservation was 27.19%, and the miscarriage rate was 34.55%, which was significantly higher than that of D5 and D6. There was little public information on the transfer of blastocysts that matured only 7 days after retrieval,<sup>[17]</sup> because slow-developing embryos are generally considered to be nonviable and are therefore discarded. Case reports of both fresh and cryopreserved embryo transfers have confirmed that blastocysts maturing as late as day 7 may still be viable.<sup>[18]</sup> The data from the present study indicated that the day 7 blastocysts could still be cryopreserved if no blastocysts were harvested on D5 or D6. In addition, patients should be informed of high miscarriage rates. During the warming cycles, the implantation rate of blastocysts decreased and the abortion rate of later development increased, which could be explained that more viable embryos would reach the expanded blastocyst

earlier.<sup>[15,19]</sup> However, another important question is, whether extended in vitro culture itself would decrease the competence of embryos to interact with endometrium? Or whether the in vivo circumstance would provide an important dynamic immune and endocrine microenvironment for blastocysts to implant better and sustain ongoing pregnancy? The longer the embryo is kept in vitro, the greater the change in its genetic traits and biological characteristics.<sup>[20]</sup> There is evidence that the blastocysts are involved in the regulation of endometrial chemokines during blastocyst attachment.<sup>[21]</sup> In the present study, the miscarriage rate of D3 embryos (23.08%) was comparable to that of blastocysts groups (D5, 15.42%; D6, 24.35%; D7, 34.55%), although the latter groups were thought to be more viable. Similar results have been found in other studies. Ana Cobo analyzed the clinical outcomes of 3150 frozen cleavage stage embryos and blastocysts. The early abortion rate on D3, D5, and D6 was 17.3%, 18.9%, and 29%,<sup>[22]</sup> respectively, consistent with the results of our study.

In the D3 embryo group of our study, the incidence of ectopic pregnancy was 4.62% after embryo transfer, while in D6 transplanted blastocysts the rate was 0.99%. Studies suggest that D3 after embryo transfer to the uterus may be a “wandering” phenomenon, which increases the ectopic pregnancy rate.

**Table 4**

**Different incubation time of blastocyst refrigerant recovery after follow-up result comparison.**

Item	D3 cleavage-stage embryo	D5 blastocyst	D6 blastocyst	D7 blastocyst
Cycles	172	721	1366	153
Clinical pregnancy	65	415	707	55
Live birth cycle	47	345	542	35
Preterm delivery cycles (rate)	8 (17.02%)	65 (18.84%)	89 (16.73%)	5 (14.29%)
Twins (rate)	15 (23.08%)a	142 (34.22%)a	211 (29.84%)a	5 (9.09%)b
Multiple births (rate)	5 (7.70%)a	6 (1.45%)b	9 (1.27%)b	1 (1.82%)ab
Gestational age	38 ± 2 weeks	38 weeks 1 day	38 weeks	38 weeks
	0 day	± 2 weeks	3 day	4 day
		2 day	± 2 weeks	± 1 weeks
			1 day	6 day
Birth sex ratio	103:100	126:100	119:100	105:100
Male baby birth weight	3.03 ± 0.99a	2.98 ± 0.82ab	3.21 ± 0.70ac	3.33 ± 0.77a
Female baby birth weight	2.82 ± 0.71a	2.96 ± 0.62ab	3.15 ± 0.58ac	3.00 ± 0.68a
Live birth	59 (30,29)	447 (250,197)	665 (361,304)	41 (21,20)
Tiny deformity		4	5	0
Severe deformity	2*	6†	6	1
Total birth defects (rate)	2 (3.39%)	10 (2.24%)	10 (1.50%)	1 (2.44%)

a.b.c.: Statistically different between different letters in the peer ( $P < .05$ ).

\* Double egg twins: 1 cleft lip and 1 cleft palate.

† With double egg twins of adrenal cortex hyperplasia.

Tiny deformities include: hernia, congenital laryngeal stridor, skin hemangioma.

Severe deformities include: congenital heart disease, nervous system abnormalities, cleft lip and palate, adrenal cortex hyperplasia, bladder fistula, Downs syndrome.

There was no significant difference in the sex ratio at birth on different cultivated days after embryo transfer. The sex ratio of boys and girls at birth in the cleavage stage group and the blastocyst groups were 103:100 and 121:100, respectively. The follow-up results showed that the proportion of boys in fresh blastocyst was 58.5% compared with 50.3% for frozen blastocyst boys after transplantation.<sup>[23]</sup> Another study on fresh and frozen blastocysts showed that during the frozen cycles, the proportion of boys was 34.4% compared with 55% for fresh transplanted blastocysts.<sup>[24]</sup> In this study, frozen surplus embryos after transplantation of D3 embryos continued to cultivate into high-quality blastocysts or all embryos were grown into blastocysts.

There was a statistically significant difference in the birth weight of babies between the D5 and D6 groups. The reason may be higher rates of the multiple rate and a higher incidence of preterm labor in the D5 blastocyst groups. As a result, the ratio of D7 blastocyst twin was lower than that of the other blastocysts twins ( $P < .05$ ) and the birth weight was slightly higher. The literature reported that the birth defect rate of frozen blastocysts was approximately 1.0% (1/103), with the birth defect rate of slow freezing cleavage stage embryos being 4.1% (8/194).<sup>[25]</sup> In this study, the vitrification D3 frozen embryos birth defect rate was slightly higher, but there was no significant difference with or between the blastocyst groups.

Some limitations of this study need to be addressed. First, although the sample size was large, all embryos were from patients admitted to the same hospital, which may limit generalization of the current findings to other areas in China or other countries. Secondly, other genetic or environmental factors that may affect embryo vitrification were not analyzed in this study, which may lead to bias to the results.

In conclusion, the implantation rate of blastocyst transfer was higher than that of staged embryo transfer and decreased ectopic pregnancy. The later developing blastocysts transfer after warming resulted in reduced implantation and increased early abortion rate. In any case, the goal of reproductive medicine practitioners is to improve the ART effect in bringing home infant rates. However, the best outcome of current artistic practice is the single birth production, not multiple babies. Finally, with the continuous improvement of the culture system in vitro, blastocyst vitrification combined with single blastocyst transplantation has become an efficient human ART therapy program.

## Author contributions

**Conceptualization:** Lizhen Xu, Rong Tang.

**Data curation:** Jingjing Jiang, Lizhen Xu, Mei Sun, Rong Tang, Shanshan Gao, Yan Sheng.

**Formal analysis:** Jingjing Jiang, Lizhen Xu, Mei Sun, Rong Tang, Shanshan Gao, Yan Sheng.

**Writing – original draft:** Rong Tang.

**Writing – review & editing:** Jingjing Jiang, Lizhen Xu, Mei Sun, Rong Tang, Shanshan Gao, Yan Sheng.

## References

- Andersen AN, Goossens V, Ferraretti AP, et al. Assisted reproductive technology in Europe, 2004: results generated from European registers by ESHRE. *Hum Reprod* 2008;23:756–71.
- Alvigi C, Conforti A, Carbone IF, Borrelli R, de Placido G, Guerriero S. Influence of cryopreservation on perinatal outcome after blastocyst- vs cleavage-stage embryo transfer: systematic review and meta-analysis. *Ultrasound Obstet Gynecol* 2018;51:54–63.
- Blake D, Farquhar C, Johnson N, Proctor M. Cleavage stage versus blastocyst stage embryo transfer in assisted conception. *Cochrane Database Syst Rev* 2007;4:CD002118.
- Martins WP, Nastro CO, Rienzi L, van der Poel SZ, Gracia C, Racowsky C. Blastocyst vs cleavage-stage embryo transfer: systematic review and meta-analysis of reproductive outcomes. *Ultrasound Obstet Gynecol* 2017;49:583–91.
- Papanikolaou EG, Kolibianakis EM, Tournaye H, et al. Live birth rates after transfer of equal number of blastocysts or cleavage-stage embryos in IVF. A systematic review and meta-analysis. *Hum Reprod* 2008;23:91–9.
- Zhao J, Xu B, Zhang Q, Li YP. Which one has a better obstetric and perinatal outcome in singleton pregnancy, IVF/ICSI or FET?: a systematic review and meta-analysis. *Reprod Biol Endocrinol* 2016;14:51.
- Tong GQ, Cao SR, Wu X, et al. Clinical outcome of fresh and vitrified-warmed blastocyst and cleavage-stage embryo transfers in ethnic Chinese ART patients. *J Ovarian Res* 2012;5:27.
- Aflatoonian A, Mansoori Moghaddam F, Mashayekhy M, Mohamadian F. Comparison of early pregnancy and neonatal outcomes after frozen and fresh embryo transfer in ART. *J Assist Reprod Genet* 2010;27:695–700.
- Menezo Y. Cryopreservation of IVF embryos: which stage? *Eur J Obstet Gynecol Reprod Biol* 2004;113(Suppl 1):S28–32.
- Mangalraj AM, Muthukumar K, Aleyamma T, Kamath MS, George K. Blastocyst stage transfer vs cleavage stage embryo transfer. *J Hum Reprod Sci* 2009;2:23–6.
- Kaur P, Swarankar ML, Maheshwari M, Acharya V. A comparative study between cleavage stage embryo transfer at day 3 and blastocyst stage transfer at day 5 in in-vitro fertilization/intra-cytoplasmic sperm injection on clinical pregnancy rates. *J Hum Reprod Sci* 2014;7:194–7.
- Wang SS, Sun HX. Blastocyst transfer ameliorates live birth rate compared with cleavage-stage embryos transfer in fresh in vitro fertilization or intracytoplasmic sperm injection cycles: reviews and meta-analysis. *Yonsei Med J* 2014;55:815–25.
- Sills ES, Palermo GD. Human blastocyst culture in IVF: current laboratory applications in reproductive medicine practice. *Rom J Morphol Embryol* 2010;51:441–5.
- Buster JE, Bustillo M, Rodi IA, et al. Biologic and morphologic development of donated human ova recovered by nonsurgical uterine lavage. *Am J Obstet Gynecol* 1985;153:211–7.
- Bourgain C, Devroey P. The endometrium in stimulated cycles for IVF. *Hum Reprod Update* 2003;9:515–22.
- Nawroth F, Rahimi G, Isachenko E, et al. Cryopreservation in assisted reproductive technology: new trends. *Semin Reprod Med* 2005;23:325–35.
- Kovalevsky G, Carney SM, Morrison LS, Boylan CF, Neithardt AB, Feinberg RF. Should embryos developing to blastocysts on day 7 be cryopreserved and transferred: an analysis of pregnancy and implantation rates. *Fertil Steril* 2013;100:1008–12.
- Desai N, Ploskonka S, Goodman L, et al. Delayed blastulation, multinucleation, and expansion grade are independently associated with live-birth rates in frozen blastocyst transfer cycles. *Fertil Steril* 2016;106:1370–8.
- Glujovsky D, Blake D, Farquhar C, Bardach A. Cleavage stage versus blastocyst stage embryo transfer in assisted reproductive technology. *Cochrane Database Syst Rev* 2012;CD002118.
- Dar S, Lazer T, Shah PS, Librach CL. Neonatal outcomes among singleton births after blastocyst versus cleavage stage embryo transfer: a systematic review and meta-analysis. *Hum Reprod Update* 2014;20:439–48.
- Mirkin S, Nikas G, Hsiu JG, Diaz J, Oehninger S. Gene expression profiles and structural/functional features of the peri-implantation endometrium in natural and gonadotropin-stimulated cycles. *J Clin Endocrinol Metab* 2004;89:5742–52.
- Cobo A, de los Santos MJ, Castellò D, Gámiz P, Campos P, Remohí J. Outcomes of vitrified early cleavage-stage and blastocyst-stage embryos in a cryopreservation program: evaluation of 3150 warming cycles. *Fertil Steril* 2012;98:1138–46.
- Takahashi K, Mukaida T, Goto T, Oka C. Perinatal outcome of blastocyst transfer with vitrification using cryoloop: a 4-year follow-up study. *Fertil Steril* 2005;84:88–92.
- Lin PY, Huang FJ, Kung FT, Wang LJ, Chang SY, Lan KC. Comparison of the offspring sex ratio between cleavage stage embryo transfer and blastocyst transfer. *Taiwan J Obstet Gynecol* 2010;49:35–9.
- Wang X, Du M, Guan Y, Wang B, Zhang J, Liu Z. Comparative neonatal outcomes in singleton births from blastocyst transfers or cleavage-stage embryo transfers: a systematic review and meta-analysis. *Reprod Biol Endocrinol* 2017;15:36.