

Associations between a new day 4 embryo grading system and implantation rates in frozen embryo transfer (FET) cycles

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Abstract

The purpose of this study is to present a new day 4 (D4) embryo grading system for the assessment of embryos in frozen-thawed embryo transfer (FET) cycles.

A new grading system (grades A–E) was modified from the 2011 ESHRE Istanbul Consensus for D4 embryos in FET cycles. In total, we retrospectively analyzed 5640 embryos with known implantation data after D4 transfer in FET cycles by using this proposed grading model.

The transferred embryos exhibited a similar declining trend in implantation rates from the top grade A to the lowest grade E. The implantation rates of grade B and E embryos in the in vitro fertilization group were significantly higher than that in the intracytoplasmic sperm injection group (grade B: 41.82%, 35.23%, $\chi^2 = 5.85$, $P < .05$ and grade E: 18.53%, 14.81, $\chi^2 = 76.86$, $P < .01$, respectively). The receiver operating characteristic analysis showed that our proposed model predicted the implantation outcomes of all embryos (area under the ROC curve = 0.65; 95% CI, 0.63–0.66; $P < .01$).

This study demonstrated that the new grading system provided by us turned out to be a useful tool in assisting embryo selection via embryo morphological changes, and D4 embryo transfer provided a simple and applicable method for a daily routine in FET cycles.

Abbreviations: FET = frozen-thawed embryo transfer, FSH = follicle-stimulating hormone, LH = luteinizing hormone, ICSI = intracytoplasmic sperm injection.

Keywords: compaction, embryo, grading system, implantation

1. Introduction

The developments of in vitro fertilization-embryo transfer technology have witnessed significant advances since the first in vitro fertilization (IVF) baby was born in the 1970s. Although there are continuous improvements in the technology of embryo

culture in vitro, there is still a lack of objective and accurate methods to judge the embryo quality as an important factor, thereby affecting the outcome of clinical pregnancy.^[1,2]

In 2011, an expert meeting from Alpha and ESHRE resulted in a consensus paper on morphological criteria for embryo assessment. However, there is no general agreement on the quality classification of day 4 (D4) embryos in the frozen embryo transfer (FET) cycles. Because embryos were usually frozen on the third day, we routinely cultured embryos overnight for transfer on the fourth day during FET cycles. It was a great challenge to select the most viable and potential embryos to transfer for D4 embryos in reducing the number of transplanted embryos while improving the ability of embryo implantation. The objective of this study is to present a novel and practical grading system for D4 embryos which could be applicable in clinical practice conceived via IVF and intracytoplasmic sperm injection (ICSI) for predicting the implantation potential of embryos in FET cycles.

2. Material and methods

This study is a single-center retrospective cohort analysis, which includes the medical records of patients with infertility taking IVF–ICSI cycles and subsequent FET from January 2015 to October 2019. The inclusion criteria for patients were as follows: female age < 40 years old, first controlled ovarian stimulation cycle in lifetime. The ethics research committee at the first hospital of Lanzhou University approved the conduct of this study.

2.1. Study group

This study included patients who received embryo transfer on D4 of FET cycles. The day 3 embryos were thawed and cultured

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This study received the approval of the Ethics Committee of the first hospital of Lanzhou university (LDYYLL2019-42).

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.

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Table 1
Demographic characteristics of IVF and ICSI groups.

	IVF	ICSI	P value
Total no. of COH cycles	1353	902	
Total no. of FET cycles	2073	1375	
Age of female	31.69 ± 5.26	31.57 ± 5.88	NS
AMH level (ng/mL)	3.80 ± 2.66	3.51 ± 2.72	NS
E2 on trigger day (pg/mL)	2294.49 ± 1044.12	2347.55 ± 1119.81	NS
P4 on trigger day (ng/mL)	1.04 ± 0.36	0.98 ± 0.41	NS
No. of oocytes retrieved	14.61 ± 6.09	13.78 ± 5.61	NS
No. of MII oocytes retrieved	12.67 ± 5.24	11.91 ± 5.27	NS
No. of embryo transferred in FET cycles	1.62 ± 0.44	1.66 ± 0.46	NS

Values are expressed as numbers, mean ± standard deviation, or percentages.

AMH = anti-müllerian hormone, E2 = serum estradiol, ET = embryo transfer, ICSI = intracytoplasmic sperm injection, MII = metaphase II, P4 = serum progesterone, IVF = in vitro fertilization, COH, controlled ovarian stimulation.

overnight. Then, 1 or 2 embryos were transferred selectively. Thereafter, we assessed whether all the embryos were implanted successfully or not. We excluded the cycles with partial implantation.

In total, 2255 patients with 3448 FET cycles were recruited for the final analysis. Table 1 shows baseline characteristics. There were 1256 cycles of single embryo transfer and 2192 cycles received 2 embryos transfer on D4. According to embryos compaction and different blastomere number ratio (BNR, number of embryo blastomere on day 4/ number of embryo blastomere on day 3, D4/D3), we calculated the implantation rates of different graded D4 embryos.

2.2. Ovarian stimulation

Patients underwent controlled ovarian stimulation in long or short protocols with GnRH agonists (Decapeptyl, Ferring, Germany) and recombinant follicle-stimulating hormone (FSH) (Puregon, Organon, the Netherlands). FSH dosage was adjusted according to the ovarian response, which was evaluated by the assessment of ultrasound and estrogen and progesterone levels. Oocyte maturation was induced with 5000 to 10,000 units of human chorionic gonadotropin (Pregnyl, Saint-Prex, Switzerland). Follicular aspiration guided by ultrasound was retrieved 36 hours after the induction of ovulation.

2.3. Embryo culture

The choice of fertilization method depended on the infertility of substantial male factor. Fertilization status was evaluated at day 1 (18 hours after insemination) for the appearance of 2 polar bodies and 2 pronucleus. Embryos were then cultured with Vitrolife G-I plus Cleavage Medium (Vitrolife, Goteborg, Sweden) in a CO₂ incubator (37°C, 6% CO₂, and 5% O₂). Embryonic development was assessed on day 3 graded with the Gardner method.^[3] All embryos were cultured to the D3 stage and vitrified by using the VitriFreeze Media Kit (Kitazato, Japan) and after thawed with VitriThaw Media Kit (Kitazato, Japan). All samples were stored in liquid nitrogen.

2.4. D4 embryo grading

We established a new grading system modified from the 2011 ESHRE Istanbul Consensus for D4 embryos in FET cycles.^[4]

Table 2
Human embryo grading classification of day 4.

Classification	Compaction	Blastomere number ratio (D4/D3)
Grade A	Full compaction	
Grade B	Part of compaction	
Grade C	No compaction	≥1.5
Grade D	No compaction	≥1.2, <1.5
Grade E	No compaction	≥1.0, <1.2

Usually, the compaction phenomenon took place during the fourth day of human embryo development. Embryos with complete compaction will be rated as grade A. Embryos with partial compaction will be classified as grade B. In our grading system, we divided embryos without compaction into grades C, D, and E according to the different BNRs (D4/D3). Embryos with BNR ≥1.5 were defined as grade C, embryos with BNR ≥1.2, <1.5 were considered as grade D, and while those with BNR ≥1.0, <1.2 were placed in grade E (Table 2). Two embryologists with at least 4 years working experience perform embryo grading at the same time.

2.5. Frozen cycle endometrial preparation

2.5.1. Artificial hormone replacement. The cycle started on day 3, in which the patients were orally administered with 2 to 6 mg of estradiol (Estrace; Allergan Pharmaceuticals) for endometrial preparation. An ultrasound endometrial assessment was conducted to assess the lining for its readiness for the ET procedure. The thickness of the endometrium was evaluated weekly until it was ≥7 mm thick. Based on the proposed day of thawing and transfer of the embryo, the progesterone suppository (200 mg, 3 times daily, tid) was vaginally administered to the women to start luteal support. The embryos were thawed on day 3 of progesterone and transferred overnight culture.

2.5.2. Natural cycles. After spontaneous menstruation, endometrial thickness and the follicular development of patients were monitored regularly by the serial ultrasound procedures. The levels of progesterone and luteinizing hormone (LH) were observed until the LH peak appeared corresponding to a day before oocyte ovulation (LH level exceeded 180% of baseline). On the second day, progesterone suppositories (200 mg tid) started to show their effects. The embryos were thawed on day 3 of progesterone administration and transferred after overnight culture.

2.5.3. Embryo transfer and confirmation of implantation.

Based on the patient's age and anamneses, 1 or 2 embryos were thawed and cultured overnight for transplantation during the natural cycle or hormone replacement FET cycle. All ET procedures were performed under ultrasonic guidance by using specified standardized techniques. Clinical pregnancy was confirmed by ultrasound visualization of the gestational sac and the detection of fetal heartbeat under ultrasound at 7 weeks of pregnancy. The total number of fetal heart activity was equal to the total number of transplanted embryos, which was considered complete implantation. Monozygotic twins are excluded.

2.6. Statistical analysis

We performed statistical analysis by using the IBM Statistical Package for the Social Sciences (IBM SPSS v.22; IBM Corporation). Continuous variables were expressed as mean values with standard deviation and analyzed by Student *t* test. Categorical

Table 3
Embryo implantation rates of IVF and ICSI.

Day 4 grading	Implantation rate (%)		χ^2	P value
	IVF	ICSI		
A	236/445 (53.03)*	147/297 (49.49)†	2.39	.12
B	327/782 (41.82)*	192/545 (35.23)†	5.85	.02
C	178/631 (28.21)*	108/420 (25.71)†	0.79	.37
D	156/685 (22.77)*	95/453 (20.97)†	0.52	.47
E	151/815 (18.53)*	84/567 (14.81)†	76.86	<.01
Total	1048/3358 (31.21)	626/2282 (27.43)	10.87	<.01

Note: values are expressed as numbers (%).
 IVF = in vitro fertilization, ICSI = intracytoplasmic sperm injection.
 * $P < .01$, $\chi^2 = 191.10$.
 † $P < .01$, $\chi^2 = 76.78$.

variables were compared by using a χ^2 test. The area under the ROC curve (AUC) was used to test the predictive ability of the receiver operating characteristic (ROC) on the outcome of embryo implantation. A P value of less than .05 was considered statistically significant for this study.

3. Results

3.1. Demographic data and cycle characteristics

Our study included 2255 controlled ovarian hyperstimulation cycles (1353 cycles in IVF group and 902 cycles in ICSI group). The baseline characteristics and demographics, including female age, anti-müllerian hormone level, E2 and P4 on trigger day, number of metaphase II oocytes retrieved, between the IVF and ICSI groups did not had a statistically significant difference (Table 1).

3.2. Implantation rates of the proposed model

In total, 5640 transferred embryos were retrospectively annotated into 5 grades (A–E) in FET cycles as described in Table 2. Table 3 shows the implantation rates of different embryo grades (embryo implantation rates of IVF and ICSI), whereas Table 4 shows the implantation rates of embryos according to age of female. Among IVF, ICSI, nonelderly and elderly groups, transferred embryos exhibited a similar declining trend in the implantation rates from the top grade to the lowest grade E ($P < .01$ for all groups). After converting the grades (from E to A) to numeric ranking (from 1 to 5), the ROC analysis showed that the proposed model predicted implantation outcomes in a degree in the IVF group (AUC=0.65; 95% CI, 0.63–0.67; $P < .01$), ICSI group (AUC=0.63; 95% CI, 0.60–0.65; $P < .01$), nonelderly

Table 4
Implantation rates of embryos according to age of female.

Day 4 grading	Implantation rate (%)		χ^2	P value
	Age <36 yr	Age ≥36 yr		
A	292/520 (56.15)*	91/222 (40.99)†	25.86	<.01
B	423/953 (44.39)*	96/374 (25.67)†	39.52	<.01
C	213/675 (31.56)*	73/376 (19.41)†	17.97	<.01
D	180/716 (25.14)*	71/422 (16.82)†	10.68	<.01
E	172/832 (20.67)*	63/550 (11.45)†	19.94	<.01
Total	1280/3696 (34.63)	394/1944 (20.27)	136.60	<.01

Values are expressed as numbers (%).
 * $P < .01$, $\chi^2 = 215.90$.
 † $P < .01$, $\chi^2 = 56.19$.

group (AUC=0.65; 95% CI, 0.63–0.67; $P < .01$), elderly group (AUC=0.64; 95% CI, 0.61–0.67; $P < .01$), or a combination of all embryos (AUC=0.65; 95% CI, 0.63–0.66; $P < .01$).

4. Discussion

The primary objective of IVF is to select the best quality embryos for transfer to achieve a healthy single pregnancy. As increasingly global consensus has been achieved to avoid multiple pregnancies after IVF/ICSI–ET treatment, selective singleton ET, supported by improved embryo selection, has been promoted to maintain pregnancy rates while reducing the risk of multiple pregnancies.^[5–7]

Li et al^[8] reported a consistent success rate between morula embryo transfer on D4 and blastocyst transfer on day 5 in fresh IVF/ET cycles. Additionally, early blastulation was a useful predictor in selecting the best embryo to achieve a higher pregnancy rate in fresh eSET cycles.^[9] Here, we proposed a simple embryonic morphological grading system that was developed on a dataset of 5640 embryos transferred on D4 in FET cycles. The fate of each embryo after transplantation (implanted and nonimplanted) was clearly known. The resulting grading system was based on total or partial compaction (grades 1 or 2, respectively) and BNR (D4/D3, grade 3, 4, or 5), modified from the 2011 ESHRE Istanbul Consensus for D4 embryos.^[4] Our grading system was feasible and operable in selecting the best quality of embryos on D4, which were classified from grade 1 to 5. The embryologist needed few minutes to observe embryos cultured on D4 in routine duties. The grading system provided by us can be easily applied to daily practice. However, using implantation as endpoint for the new day 4 embryo grading system was an inherent limitation of the present study. Another limitation of the study was that body mass index (BMI) data were not collected. BMI is inversely related to embryo quality and IVF outcome.^[10,11] Furthermore, the interobserver and intraobserver variability in grading the embryos in the new day 4 embryo grading system were not determined.^[12]

There were several retrospective cohort studies at different reproductive medical centers conducted on patients who underwent in vitro fertilization with preimplantation genetic screening. Minasi et al^[13] showed that there appears to be a relationship between the polyploidy and blastocyst morphologies. Excellent- and fair-quality embryos had higher euploidy rates and a higher chance to result in an ongoing pregnancy.^[14] As described in the previous literature, embryo contraction, a physiological feature during blastulation, was conditioned by the ploidy status of the embryo. Additionally, the presence of contractions may compromise the implantation rates.^[15] Taken altogether, maternal age and some morphological parameters (expansion, time of blastocyst formation, and trophectoderm grade) were associated with euploidy, whereas other parameters, such as embryo sex and biopsy day, were not related to aneuploidy. There was a positive correlation between the speed of D4 embryo development and the time of blastocyst formation. Compaction embryos of D4 (grade 1) may have higher chromosome aneuploidy than other groups.

As compared with cleavage/blastocyst embryos transfer, there were several advantages of morula embryo transfer on D4. D4 embryos transplantation can effectively eliminate the phenomenon of embryos arrest in the cleavage stage.^[16,17] It was well known that human embryos entered the uterine cavity on the fourth day after fertilization. To some extent, D4 embryos transfer

was more natural than the cleavage embryos transfer of D3. In vitro culture time of the embryos transplanted on D4 was reduced as compared with that of the blastocysts. This reduction decreased the sensitivity of the disruption of epigenetic regulation mechanism.^[18,19] Other research works have demonstrated that morula embryo transfer and blastocyst transfer had shown compatible pregnancy rates.^[20]

This study excluded the cycles with partial implantation. The embryo implantation results of our included study were clear and provided the personalized information for each embryo. Our data showed that transferred embryos exhibited a similar declining trend in implantation rates from the top grade to the lowest grade E. The implantation rates of grade B and E embryos in the IVF group were significantly higher than that in the ICSI group, thereby suggesting that ICSI fertilization may have a certain impact on embryo development potential. The implantation rates of grade A and grade B embryos in the nonelderly group were 56.15% and 44.39%, respectively. Thus, selective D4 single embryo transfer may be attempted in the nonelderly group in the future during the FET cycles.

In conclusion, our new grading system is a useful tool to assist in embryo selection via embryo morphological changes, and D4 embryo transfer provides a simple and applicable method for daily routine. However, the transferability of this model between laboratories with independent patient populations and large-sized prospective randomized controlled studies are still required to be studied in future research works.

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Author contributions

Hongxing Li and Yuanxue Jing contributed to the conception of the study; Xiaojuan Xu and Hongxing Li contributed significantly to analysis and manuscript preparation; Hongxing Li and Lin Liu performed the data analyses and wrote the manuscript; Di Cao and Yiqing Wang helped perform the analysis with constructive discussions.

Corrections

When originally published, Dr. Lin Liu's name appeared incorrectly as Ling Liu and has since been corrected.

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