A predictive model for high-quality blastocyst based on blastomere number, fragmentation, and symmetry

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Abstract

Purpose The aim of this study was to create a predictive model for high-quality blastocyst progression based on the traditional morphology parameters of embryos.

Methods A total of 1564 embryos from 234 women underwent conventional in vitro fertilization and were involved in the present study. High-quality blastocysts were defined as having a grade of at least 3BB, and all embryos were divided based on the development of high-quality blastocysts (group HQ) or the failure to develop high-quality blastocysts (group NHQ). A retrospective analysis of day-3 embryo parameters, focused on blastomere number, fragmentation, the presence of a vacuole, symmetry, and the presence of multinucleated blastomeres was conducted.

Results All parameters were related to high-quality blastocysts ($p < 0001$) in t tests, chi-square tests, or Fisher tests. The individual scores for all parameters were determined according to their distributions and corresponding rates of forming high-quality blastocysts. Parameters are indicated by s_bn (blastomere number), s_f (fragmentation), s_pv (presence of a vacuole), s_s (symmetry), and s_MNB (multinucleated blastomeres). Subsequently, univariate and multivariate logistic regression analyses were conducted to explore their relationship. In the multivariate logistic regression analysis, a predictive model was constructed, and a parameter Hc was created based on the s_bn, s_f, and s_s parameters and their corresponding odds ratios. The value of Hc in group HQ was significantly higher than that in group NHQ. A receiver operating characteristic curve was used to test the effectiveness of the model. An area under the curve of 0.790, with a 95% confidence interval of 0.766–0.813, was calculated. A dataset was used to validate the predictive utility of the model. Moreover, another dataset was used to ensure that the model can be applied to predict the implantation of day-3 embryos.

Conclusions A predictive model for high-quality blastocysts was created based on blastomere number, fragmentation, and symmetry. This model provides novel information on the selection of potential embryos.

Keywords In vitro fertilization · Embryo development · Blastocyst · Embryo evaluation

Introduction

The morphological assessment of embryos has been used as a tool at the beginning of in vitro fertilization (IVF); its

effectiveness has increased with the improvement of this technology [1, 2]. It has been reported that the evaluation of embryos is associated with embryo morphological parameters such as blastomere number, fragmentation, symmetry, the presence of a vacuole, and the presence of multinucleated blastomeres (MNBs) [3]. In recent decades, experts have gradually reached a consensus on the morphological assessment of embryos, demonstrating that embryo quality may be graded as good, fair, or poor based on blastomere number, fragmentation, and symmetry [3, 4].

Although embryo evaluation criteria can help us select embryos [4], scholars have further claimed that the implantation rate and pregnancy rate for blastocysts are higher than those for embryos, indicating that blastocyst transfer is more advantageous than day-3 embryo transfer, particularly for high-

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quality blastocysts [5, 6]. It has also been reported that a good day-3 embryo is apt to form a high-quality blastocyst [7, 8]. However, not all good day-3 embryos can form high-quality blastocysts, partly because of the negative effect of being exposed to in vitro conditions for a long time [9]. Prolonged culture confers no advantage if the number of embryos available is low or embryo quality is poor [9, 10]. In that case, it is worthwhile to create a predictive model for high-quality blastocyst progression to select the best day-3 embryo.

In the present study, we aim to create a predictive model for high-quality blastocyst progression based on the morphological parameters of day-3 embryos; this model will provide us with novel information for the selection of the best day-3 embryos and a reduction in prolonged culture.

Materials and methods

The present study was conducted in the Reproductive Medicine department of the Affiliated Hospital of Dali University from January 2013 to December 2016. A total of 1564 embryos retrieved from 234 women were included. All analyzed patients underwent conventional IVF treatment; patients who underwent intracytoplasmic sperm injection (ICSI) and patients with male factor infertility were excluded. This study was approved by the Institutional Review Board of Dali University.

Gonadotropin-releasing hormone agonist (GnRH-a; buserelin acetate, Suprefact; Hoechst, Frankfurt, Germany) and recombinant follicle-stimulating hormone (rFSH; Gonal-f, Modugno, Italy) were used for the long protocol of ovarian stimulation [11]. When at least three follicles reached a 17-mm diameter or at least two follicles reached an 18-mm diameter, recombinant hCG (250 mg; Ovidrel; Serono) was administered to trigger ovulation, and oocytes were retrieved 36 h after hCG injection [12].

Insemination was performed 4 h after oocyte collection; 16 h later, fertilization was checked, and zygotes with two pronuclei (2PN) were considered normally fertilized eggs. Subsequently, fertilized oocytes were cultured in early cleavage medium (Irvine Scientific, CA, USA) for 3 days. On the mornings of day 2 and day 3, multilevel images of embryos were recorded using laser software (ZILOS-tk Laser; Hamilton Thorne

Research, USA). Subsequently, blastomere number, embryos with/without MNBs, and embryos with/without the presence of a vacuole were observed and recorded, and the extent of fragmentation and symmetry was calculated based on previous findings [13]. A perfectly symmetrical embryo was defined as having less than 25% difference among blastomeres [14]. On the morning of day 3, one or two embryos were frozen, and the remaining embryos were transferred to a continuous single culture medium (Irvine Scientific, CA, USA). The growth stage, inner cell mass (ICM), and trophoctoderm (TE) of blastocysts were assessed according to the findings of Gardner and Schoolcraft [15]. ICM and TE were evaluated only when the growth stage was at least full blastocyst (3/4/5 stage). A high-quality blastocyst was defined as having a grade of at least 3BB, including 3/4/5AA, AB, BA, or BB [16, 17]. All lab operations for gametes, zygotes, and embryos were conducted by two embryologists with considerable intra- and inter-observer variability in quality control proficiencies. The evaluation results were recorded when their results were the same, or the embryologists would discuss and determine the final results. A single, best, and fresh blastocyst was transferred into the patient's uterine cavity on day 5 or 6, and the remaining blastocysts were singly cryopreserved, or all blastocysts of some patients were singly cryopreserved. The best blastocysts selected according to the data of patient blastocysts were singly thawed and transferred according to patient needs. The luteal phase was supported by the administration of crinone gel and utrogestan for at least 14 days [18]. The β -hCG level was tested on the 13th day following embryo transfer. Clinical pregnancy was determined by the presence of a gestational sac with a fetal heartbeat rate after 6 weeks of gestation, and the implantation rate was defined as the ratio of gestational sacs to transferred embryos. Monozygotic twins were defined as having one gestation sac [18].

Based on the parameter distributions and the formation rate of high-quality blastocysts, an “individual score” was assigned to each case, comprising blastomere number, fragmentation, symmetry, the presence of a vacuole, and the presence of MNBs, which are indicated by *s_bn*, *s_f*, *s_s*, *s_pv*, and *s_MNBs*, respectively. Details for individual scores are presented in Table 1.

Statistical Package for Social Sciences 17.0 (SPSS Inc., Chicago, IL, USA) was used for statistical analysis.

Table 1 The individual scores for all analyzed morphology parameters

Parameters	Blastomere number (<i>n</i>)								Fragmentation degree (%)				Symmetry		Presence of vacuole		Presence of MNBs	
	≤3	4	5	6	7	8	9	≥10	≤5	6–10	11–15	≥15	Equal	Unequal	Yes	No	Yes	No
DHQB rate (%)	4.3	12.2	13.3	20.3	42.9	55.6	40.9	33.7	45.2	25.0	12.3	9.4	37.4	15.8	11.9	33.9	0.0	33.8
Individual score	0	1	1	2	4	5	4	3	5	3	1	0	1	0	0	1	0	1

DHQB, development to high-quality blastocyst; MNBs, multinucleated blastomeres

Fig. 1 Four types of high-quality expanded blastocysts. Blastocyst evaluation was based on Gardner et al. A high-quality blastocyst was defined as having a grade of at least 3BB. Four types of high-quality expanded blastocysts were 4AA, 4AB, 4BA, and 4BB. **a** 4AA, **b** 4AB, **c** 4BA, and **d** 4BB. ICM indicates the inner cell mass, and TE indicates the trophoctoderm

Quantitative variables are expressed as the mean and standard deviation ($M \pm SD$) and were analyzed using t tests. Categorical data are expressed as the number or percentage and were tested by chi-square tests (χ^2 test) or Fisher tests. Subsequently, univariate logistic regression analysis was performed to explore the relationship between morphology parameters and high-quality blastocysts. Multivariate logistic regression analysis was conducted to construct a predictive model based on odds ratios (ORs), and a receiver operating characteristic (ROC) curve was used to test the effectiveness of the model. Moreover, one dataset was analyzed to validate the predictive utility of the model, and the other dataset was used to determine whether the model can be applied to predict the implantation of day-3 embryos. All datasets were from the same center. The level of significance was set at $p < 0.05$.

Results

Of 1564 embryos, 521 developed into high-quality blastocysts (group HQ), whereas 1043 embryos formed poor-quality blastocysts or arrested before the blastocyst stage (group NHQ). The representative results for high-quality expanded

blastocysts are presented in Fig. 1. The mean age of the women was 29.69 ± 3.83 years (21–43 years), the mean infertility duration was 4.2 ± 2.83 years (1–12 years), the mean dose of gonadotrophins was 1969.50 ± 663.12 IU, the mean use day was 10.40 ± 1.62 days, and a mean number of 13.57 ± 4.77 oocyte pieces were retrieved.

Fig. 2 Differences in day-3 blastomere number and fragmentation between two groups. Double asterisk indicates a significant difference ($p < 0.001$) between groups NHQ and HQ. Group NHQ was the group that failed to form high-quality blastocysts. Group HQ was the group that formed high-quality blastocysts

Fig. 3 Differences in the presence of a vacuole and the presence of multinucleated blastomeres (MNBs) and symmetry between the two groups. Double asterisk indicates a significant difference ($p < 0.001$) between the two groups. **a** the difference in the presence of a vacuole between the two groups, **b** the difference in the presence of multinucleated blastomeres between the two groups, **c** the difference in symmetry between the two groups. Group NHQ was the group that failed to form high-quality blastocysts. Group HQ was the group that formed high-quality blastocysts

The implantation rates and clinical pregnancy rates for fresh and frozen-thawed blastocysts (first or second embryo transfer cycle only) were analyzed in both groups. In group HQ, 125 fresh blastocysts were transferred, and 73 gestational sacs were observed; 51 frozen-thawed blastocysts were transferred, and 33 gestational sacs (two of these were one set of monozygotic twins) were identified. Additionally, 45 fresh blastocysts and 40 frozen-thawed blastocysts from group NHQ were transferred, with the implantations of 11 and 13 blastocysts (no monozygotic twins), respectively. The implantation rates and clinical pregnancy rates of fresh blastocysts (58.4 vs. 24.4%, $p < 0.001$) and frozen-thawed blastocysts (62.7 vs. 32.5%, $p = 0.004$) in group HQ were significantly higher than those in group NHQ.

The embryonic parameter data were analyzed between group NHQ and group HQ. Consequently, significant

differences were observed in blastomere number, fragmentation, symmetry, the presence of vacuoles, and the presence of MNBs (Figs. 2 and 3). Blastomere number (7.7 ± 2.6 vs. 6.3 ± 2.9 , $p < 0.001$) and the frequency of embryos with perfect symmetry (91.0 vs. 75.9%, $p < 0.001$) in group HQ were significantly higher than those in group NHQ, whereas the extent of fragmentation (4.1 ± 8.2 vs. $10.8 \pm 13.5\%$, $p < 0.001$) and the frequency of embryos with vacuole (1.0 vs. 3.5%, $p < 0.001$) and embryos with MNBs (0.0 vs. 2.1%, $p < 0.001$) were significantly decreased in group HQ compared with those in group NHQ.

On the basis of the s_{bn} , s_f , s_s , s_{pv} , and s_{MNBs} parameters, univariate logistic regression analysis was conducted to evaluate the effects of all the above parameters on the formation of high-quality blastocysts. The results indicated that the s_{bn} (beta coefficient [B] = 0.553, standard error

Table 2 Results of univariate logistic regression analyses

Variable	<i>B</i>	<i>SE</i>	Wald	Odds ratio	95% confidence interval		<i>p</i> value
s_{bn}	0.553	0.039	203.683	1.739	1.612	1.876	< 0.001
s_f	0.428	0.038	129.485	1.533	1.425	1.651	< 0.001
s_{pv}	− 1.334	0.480	7.737	0.263	0.103	0.674	0.005
s_{MNBs}	− 2.369	1.025	5.342	0.094	0.013	0.698	0.021
s_s	1.162	0.169	47.153	3.196	2.294	4.453	< 0.001

B, beta coefficient; *SE*, standard error; s_{bn} , the individual day-3 blastomere number score; s_f , the individual fragmentation degree score; s_{pv} , the individual presence of vacuole score; s_{MNBs} , the individual presence of multinucleated blastomere score; s_s , the individual symmetry score

Table 3 Multivariate logistic regression model in relation to high-quality blastocysts

Variable	<i>B</i>	<i>SE</i>	Wald	Odds ratio	95% confidence interval		<i>p</i> value
<i>s_bn</i>	0.464	0.041	130.908	1.590	1.469	1.722	< 0.001
<i>s_f</i>	0.335	0.041	68.009	1.399	1.291	1.515	< 0.001
<i>s_s</i>	1.223	0.180	46.338	3.397	2.389	4.830	< 0.001

B, beta coefficient; *SE*, standard error; *s_bn*, the individual day-3 blastomere number score; *s_f*, the individual fragmentation degree score; *s_s*, the individual symmetry score

[*SE*] = 0.039, wald = 203.683, $p < 0.001$, OR = 1.739), *s_f* ($B = 0.428$, *SE* = 0.038, wald = 129.485, $p < 0.001$, OR = 1.533), *s_pv* ($B = -1.334$, *SE* = 0.480, wald = 7.737, $p = 0.005$, OR = 0.263), *s_MNBs* ($B = -2.369$, *SE* = 1.025, wald = 5.342, $p = 0.021$, OR = 0.094), and *s_s* ($B = 1.162$, *SE* = 0.169, wald = 47.153, $p < 0.001$, OR = 3.196) parameters were significantly associated with high-quality blastocysts (Table 2). Importantly, the OR values for *s_bn*, *s_f*, and *s_s* were much higher than those for *s_pv* and *s_MNBs*.

Based on the univariate logistic regression analysis results, the *s_bn*, *s_f*, and *s_s* parameters were considered in the multivariate logistic regression analysis. Consequently, the *B*, *SE*, wald, OR, and *p* values of the *s_bn*, *s_f*, and *s_s* parameters were 0.464, 0.041, 130.908, 1.590, and < 0.001; 0.335, 0.041, 68.009, 1.399, and < 0.001; and 1.223, 0.180, 46.338, 3.397, and < 0.001, respectively (Table 3). According to these parameters (*s_bn*, *s_f*, and *s_s*) and the corresponding OR values, parameter Hc was created and calculated using Eq. 1.

$$Hc = s_bn * OR_{s_bn} + s_f * OR_{s_f} + s_s * OR_{s_s}. \quad (1)$$

Every embryo was assigned an Hc value according to Eq. 1. As presented in Fig. 4, the Hc value for embryos in group HQ was significantly higher than that in group NHQ (15.5 ± 3.4 vs. 11.0 ± 4.6 , $p < 0.001$). Subsequently, an ROC curve

Fig. 4 Differences in the Hc score ($p < 0.001$) between the two groups. The Hc value in group HQ was significantly higher than that in group NHQ (15.5 ± 3.4 vs. 11.0 ± 4.6 , $p < 0.001$). Group NHQ was the group that failed to form high-quality blastocysts. Group HQ was the group that formed high-quality blastocysts

was calculated to test the quality of the model as a prediction tool. The results for the ROC curve are presented in Fig. 5, with an area under the curve (AUC) of 0.790, a 95% confidence interval (CI) of 0.766–0.813, and a cut-off of 13.051; a sensitivity of 81.8% and a specificity of 64.5% were noted.

One dataset was used to validate the created model. As a result of the prolonged culture of 363 embryos, 108 high-quality blastocysts were obtained. The Hc value for the group of embryos developing into high-quality blastocysts was significantly higher than that for the group of embryos failing to form high-quality blastocysts (15.6 ± 3.1 vs. 11.3 ± 4.5 , $p < 0.001$). We obtained similar outcomes for the ROC analysis: AUC = 0.792, 95% CI from 0.743 to 0.841, and Hc cut-off of 13.464 with a sensitivity of 80.6% and a specificity of 65.1%. Moreover, 363 embryos were divided into four groups based on the range of Hc values, and a significant difference was observed in the formation rates of high-quality blastocysts among the studied groups (0.0 vs. 7.8 vs. 20.7 vs. 51.3%, $p < 0.001$). In addition, the high-quality blastocyst formation rate increased as Hc increased, as presented in Table 4.

Fig. 5 The ROC curve for the prediction of high-quality blastocysts by the Hc parameter. The area under the curve was 0.790, the standard error was 0.012, the asymptotic significance was < 0.001, the 95% confidence interval was from 0.766 to 0.813, and the cut-off for Hc was calculated as 13.051, with a sensitivity of 81.8% and a specificity of 64.5%

Table 4 Rates of development to high-quality blastocysts among ranges of Hc parameters in an independent dataset

Range (N)		Hc ≤ 4.84 (15)	4.85 ≤ Hc ≤ 9.70 (77)	9.71 ≤ Hc ≤ 14.55 (121)	Hc ≥ 14.56 (150)
Development to high-quality blastocysts	N	0	6	25	77
	%	0.0	7.8	20.7	51.3
<i>p</i> value		< 0.001			

Parameter Hc was created based on the odds ratios in the multivariate logistic regression model. $Hc = s_{bn} * OR_{s_{bn}} + s_f * OR_{s_f} + s_s * OR_{s_s}$

The other dataset was used to determine whether the model can be applied to predict the implantation of day-3 embryos. In 199 single, fresh day-3 embryo transfer cycles (the first or second embryo transfer cycle only), 62 embryos were implanted, and the implantation rate was 31.2%. The Hc value for the group of implanted embryos was significantly higher than that for the other group (16.5 ± 2.5 vs. 14.1 ± 4.5 , $p < 0.001$). The ROC results indicated an AUC of 0.657 and a 95% CI of 0.581–0.734.

Discussion

Embryo evaluation and selection of the best embryos for transfer are critical issues in assisted reproductive techniques. Over the past three decades, there have been various evaluation systems for day-3 embryos, including morphological assessment, metabolomic evaluation, and dynamic assessment of embryos with time-lapse imaging systems [1, 4, 19]. Among them, morphological assessment has been the primary and most commonly used approach [3, 4]. However, although it is much improved, morphological assessment has its own drawbacks and limitations. For example, the efficiency of morphological assessment is low for IVF treatment, and morphological assessment is easily influenced by embryologist subjectivity [3]. For these reasons, there is certainly a need for improvements in the cost-effectiveness of the method.

Generally, day-3 embryos or day-5 blastocysts are transferred in the clinic. Blastocysts are known to have higher implantation and pregnancy rates than day-3 embryos [6, 10, 20, 21]; this finding is associated with less aneuploidy [22]. Higher implantation and pregnancy rates allow the transfer of a single blastocyst to reduce the risk of multiple gestations [5, 21]. Meanwhile, the top day-3 embryos also have an optimistic pregnancy rate [8]. It has been reported that day-3 embryo transfers with more than two eight-cell-stage embryos and less than 20% fragmentation appear to have a similar implantation rate as the blastocyst transfers [23], and blastocysts do not improve the cumulative pregnancy rate [24–27]. However, the prolonged culture can eliminate a number of day-3 embryos and even create for some patients only a few poor-

quality blastocysts or no blastocyst for transfer. Prolonged culture increases the financial burden of patients, the workload of laboratories, and the mental pressure of embryologists. Therefore, it appears to be more convenient and advantageous to select the best day-3 embryos for patient pregnancy.

Blastocysts are classified into three groups: top quality (3AA, 4AA, 5AA), good quality (3/4/5 AB, BA, BB), and poor quality (3/4/5AC, CA, BC, CB, CC) [17]. Phan et al. concluded that 32% of top-quality blastocysts were aneuploid, 41% of good-quality blastocysts were aneuploid, and 80% of poor-quality blastocysts were aneuploid [28]. Gardner et al. demonstrated that the pregnancy rate for a single top-quality blastocyst was 69.6% and that the rate for two low-quality transferred blastocysts was only 43.8%, demonstrating that the pregnancy rate is related to the quality of the blastocyst [29]. In the present study, a high-quality blastocyst is defined as having a grade of at least 3BB, including top- and good-quality blastocysts. Of 261 single blastocyst transfer cycles, the clinical pregnancy rates for fresh and frozen-thawed high-quality blastocysts were 58.4 and 62.7%, respectively; the clinical pregnancy rates of fresh and frozen-thawed poor-quality blastocysts were only 24.4 and 32.5%. This observation is consistent with the findings of Gardner [29], demonstrating that the utility of the grouping and experiment was well considered and that it is meaningful for us to discuss the relations between parameters and high-quality blastocysts.

Machtinger et al. demonstrated that embryo assessment was based on blastomere number, fragmentation, and symmetry in the morphological assessment [3], and this finding was confirmed by the outcomes of univariate logistic regression in the present study. Interestingly, we constructed a model for predicting high-quality blastocysts, which indicated that day-3 embryos with higher Hc values more easily formed high-quality blastocysts. The Hc parameter appears to be a good predictor since blastomere number, fragmentation, and symmetry are more important parameters for day-3 embryos. The Hc parameter is determined by the scores and OR values of the three parameters indicated above, and the predictive power of the model was confirmed by the results of an ROC analysis (Fig. 5). Moreover, we further analyzed a dataset to validate

the effectiveness of the model, and the results were similar, suggesting that the model is repeatable and reliable. In addition, the model validated that it may be applied to predict the implantation of day-3 embryos using a dataset of 199 single-embryo transfer cycles. Therefore, the predictive model may be used as a tool in the clinic.

Currently, although the embryo morphology evaluation has been varied, there are only two standardized criteria, proposed by the Alpha Executive and European Society of Human Reproduction and Embryology Special Interest Group of Embryology and the group of the Society for Assisted Reproductive Technology (SART) [3]. For the initial criterion, cleavage stage embryos were classified into three categories, good, fair, and poor, according to the timing of observations, blastomere number, fragmentation, symmetry, and multinucleation [30]. The criterion from the SART group revealed that the quality of cleavage embryos was also assessed as good, fair, or poor, based simply on blastomere number, fragmentation, and symmetry [31]. However, it appears that those criteria were recapitulative rather than accurate. In the present study, we constructed a model and a parameter of Hc in multivariate logistic regression analysis based on blastomere number, fragmentation, and symmetry. The constructed model was similar to the standardized criterion adopted by the SART group but was not confined to that criterion. It is more accurate and easier to calculate the Hc value of day-3 embryos, and we can identify the best day-3 embryos from various embryos based on Hc values.

However, it is important to note that the present study has some limitations. First, all datasets were from the same center, and a multicenter study was also necessary to validate the corresponding model. Second, the zygote score could help us to select an embryo, and a combined evaluation of both the zygote and cleavage stage embryo is highly predictive [32]. However, the present study did not analyze the effects of the zygote on high-quality blastocysts, and we plan to construct a similar model combining the evaluations from zygotes and embryos in a follow-up study. Third and finally, live birth is the most significant endpoint, but unfortunately, we did not use this better approach.

Conclusions

Morphological assessment of embryo appearance is a classic and widely used approach to embryo evaluation; however, this approach requires gradual improvement. In the present study, we confirmed the association of high-quality blastocysts with blastomere number, fragmentation, symmetry, the presence of vacuoles, and the presence of MNBs. More importantly, we identified a novel model that exhibits a strong predictive power for high-quality blastocysts. The predictive model can be

applied to clinical practice to select potential embryos for transfer and to shorten embryo incubation.

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Compliance with ethical standards

This study was approved by the Institutional Review Board of Dali University.

Conflict of interest The authors declare that they have no conflict of interest.

References

1. Milewski R, Kuć P, Kuczyńska A, Stankiewicz B, Łukaszuk K, Kuczyński W. A predictive model for blastocyst formation based on morphokinetic parameters in time-lapse monitoring of embryo development. *J Assist Reprod Genet.* 2015;32(4):571–9.
2. Motato Y, LSM J d, Escriba MJ, Meseguer M. Morphokinetic analysis and embryonic prediction for blastocyst formation through an integrated time-lapse system. *Fertil Steril.* 2015;105(2):376–84.
3. Machtinger R, Racowsky C. Morphological systems of human embryo assessment and clinical evidence. *Reprod BioMed Online.* 2013;26(3):210–21.
4. Racowsky C, Vernon M, Mayer J, Ball GD, Behr B, Pomeroy KO, et al. Standardization of grading embryo morphology. *J Assist Reprod Genet.* 2010;94(3):1152–3.
5. Adler A, Lee H, McCulloh DH, Ampeloquio E, Clarke-Williams M, Wertz BH, et al. Blastocyst culture selects for euploid embryos: comparison of blastomere and trophectoderm biopsies. *Reprod BioMed Online.* 2014;28(4):485–91.
6. Surrey ES, Schoolcraft WB. Blastocyst versus cleavage stage embryo transfer: maximizing success rates. In: Sauer MV, editor. *Principles of oocyte and embryo donation.* London: Springer; 2013. p. 129–39.
7. Sasikala N, Rajapriya A, Mahalakshmi S, Janani DM, Archana B, Parameaswari PJ. Blastocyst culture depends on quality of embryos on day 3, not quantity. *Middle East Fertil Soc J.* 2015;20(4):224–30.
8. Yin H, Jiang H, He R, Wang C, Zhu J, Luan K. The effects of fertilization mode, embryo morphology at day 3, and female age on blastocyst formation and the clinical outcomes. *Syst Biol Reprod Med.* 2015;(1):61, 1–7.
9. Minasi MG. Current aspects of blastocyst culture, biopsy and vitrification. *Curr Trends Clin Embriol.* 2014;1(1):27–33.
10. Thum MY, Wells V, Abdalla H. Patient selection criteria for blastocyst culture in IVF/ICSI treatment. *J Assist Reprod Genet.* 2010;27(9–10):555–60.
11. Anckaert E, Smits J, Schiettecatte J, Klein BM, Arce JC. The value of anti-müllerian hormone measurement in the long GnRH agonist protocol: association with ovarian response and gonadotrophin-dose adjustments. *Hum Reprod.* 2012;27(6):1829–39.
12. Huang B, Ren X, Wu L, Xu B, Li Y, et al. Elevated progesterone levels on the day of oocyte maturation may affect top quality embryo IVF cycles. *PLoS One.* 2016;11(1):e0145895.
13. Gallardo EF, Spiessens C, D’Hooghe T, Debrock S. Effect of embryo morphology and morphometrics on implantation of vitrified

- day 3 embryos after warming: a retrospective cohort study. *Reprod Biol Endocrinol*. 2016;14(1):40.
14. Paternot G, Debrock S, D'Hooghe T, Spiessens C. Computer-assisted embryo selection: a benefit in the evaluation of embryo quality? *Reprod BioMed Online*. 2011;23(3):347–54.
 15. Gardner DK, Schoolcraft WB. Towards reproductive certainty: infertility and genetics beyond. In: Jansen R, Mortimer D, editors. *In vitro culture of human blastocysts*. Carnforth: Parthenon Press; 1999. p. 378–88.
 16. Smuts MP, Miller M, Ramdas-Navale K, Aoki VW. Improved high-quality blastocyst formation rates result from in vitro embryo culture in the cook MINC incubator platform. *Fertil Steril*. 2008;89(4): Suppl 21.
 17. Cetinkaya M, Pirkevi C, Yelke H, Colakoglu YK, Atayurt Z, Kahraman S. Relative kinetic expressions defining cleavage synchronicity are better predictors of blastocyst formation and quality than absolute time points. *J Assist Reprod Genet*. 2015;32(1):27.
 18. Sang MK, Sang WL, Jeong HJ, San HY, Min WK, Jin HL, et al. Clinical outcomes of elective single morula embryo transfer versus elective single blastocyst embryo transfer in IVF-ET. *J Assist Reprod Genet*. 2012;29(5):423.
 19. Marhuenda-Egea FC, Martínez-Sabater E, Gonsálvez-Alvarez R, Lledó B, Ten J, Bernabeu R. A crucial step in assisted reproduction technology: human embryo selection using metabolomic evaluation. *Fertil Steril*. 2010;94(2):772.
 20. Azimineko E, Mohseni Salehi MS, Kalantari V, Shahrokh Tehraninejad E, Haghollahi F, Hossein Rashidi B, et al. Pregnancy outcome after blastocyst stage transfer comparing to early cleavage stage embryo transfer. *Gynecol Endocrinol*. 2015;31(11):880–4.
 21. McClelland S, Melzer K, McCulloh DH, Grifo JD. A comparison of pregnancy outcomes between day 3 and day 5/6 fresh embryo transfers: does day of embryo transfer (ET) really make a difference? *J Assist Reprod Genet*. 2015;32(2):1–6.
 22. Jaroudi S, Alfarawati S, Poli M, Wells D, Fragouli E. The effect of aneuploidy on embryo morphology and preimplantation development from the cleavage to the blastocyst stage. *Fertil Steril*. 2012;98(3):S164.
 23. Bungum M, Bungum L, Humaidan P, Andersen CY. Day 3 versus day 5 embryo transfer: a prospective randomized study. *Reprod BioMed Online*. 2003;7(1):98–104.
 24. Martins WP, Natri 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(5):583.
 25. De Vos A, Van Landuyt L, Santosribeiro S, Camus M, Van de Velde H, Tournaye H, et al. Cumulative live birth rates after fresh and vitrified cleavage-stage versus blastocyst-stage embryo transfer in the first treatment cycle. *Hum Reprod*. 2016;31(11):2442.
 26. Fernándezshaw S, Cercas R, Braña C, Villas C, Pons I. Ongoing and cumulative pregnancy rate after cleavage-stage versus blastocyst-stage embryo transfer using vitrification for cryopreservation: impact of age on the results. *J Assist Reprod Genet*. 2015;32(2):177–84.
 27. Levi-Setti PE, Cirillo F, Smeraldi A, Morengi E, Mulazzani GEG, Albani E. No advantage of fresh blastocyst versus stage embryo transfer in women under the age of 39: a randomized controlled study. *J Assist Reprod Genet*. 2017;34(11):1–9.
 28. Phan V, Littman E, Harris D, La A. Correlation between aneuploidy and blastocyst quality. *Asian Pac J Reprod*. 2014;3(4):253–7.
 29. Gardner DK, Lane M, Stevens J, Schlenker T, Schoolcraft WB. Blastocyst score affects implantation and pregnancy outcome: towards a single blastocyst transfer. *Fertil Steril*. 2000;73(6):1155.
 30. Alpha Scientists in Reproductive Medicine and ESHRE Special Interest Group of Embryology. Istanbul consensus workshop on embryo assessment: proceedings of an expert meeting. *Reprod BioMed Online*. 2011;22(6):632–46.
 31. Racowsky C, Vernon MJ, Ball GD, Behr B, Pomeroy KO, Wininger D, et al. Standardization of grading embryo morphology. *Fertil Steril*. 2010;27(8):437–9.
 32. Lan K, Huang F, Lin Y, Kung F, Hsieh C, Huang H, et al. The predictive value of using a combined Z-score and day 3 embryo morphology score in the assessment of embryo survival on day 5. *Hum Reprod*. 2003;18(6):1299.