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ARTICLE

Women's age and embryo developmental speed accurately predict clinical pregnancy after single vitrified-warmed blastocyst transfer

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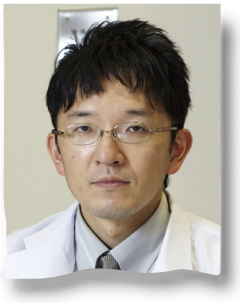

Dr. Keiichi Kato is general director at the Kato Ladies Clinic in Tokyo, Japan. Dr. Kato earned his MD from the University of Kanazawa. His main research interests are minimal ovarian stimulation, natural-cycle IVF, and single-embryo transfer.

Abstract The aim of this study was to establish a simple, objective blastocyst grading system using women's age and embryo developmental speed to predict clinical pregnancy after single vitrified-warmed blastocyst transfer. A 6-year retrospective cohort study was conducted in a private infertility centre. A total of 7341 single vitrified-armed blastocyst transfer cycles were included, divided into those carried out between 2006 and 2011 (6046 cycles) and 2012 (1295 cycles). Clinical pregnancy rate, ongoing pregnancy rate and delivery rates were stratified by women's age (<35, 35–37, 38–39, 40–41, 42–45 years) and time to blastocyst expansion (<120, 120–129, 130–139, 140–149, >149 h) as embryo developmental speed. In all the age groups, clinical pregnancy rate, ongoing pregnancy rate and delivery rates decreased as the embryo developmental speed decreased ($P < 0.0001$). A simple five-grade score based on women's age and embryo developmental speed was determined by actual clinical pregnancy rates observed in the 2006–2011 cohort. Subsequently, the novel grading score was validated in the 2012 cohort (1295 cycles), finding an excellent association. In conclusion, we established a novel blastocyst grading system using women's age and embryo developmental speed as objective parameters.

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KEYWORDS: blastocyst culture, in-vitro fertilization, minimal ovarian stimulation, outcome prediction, single embryo transfer

Introduction

Single blastocyst transfer is increasingly used during IVF treatment to prevent multiple conceptions. When several embryos are available after prolonged embryo culture, a thorough evaluation of each blastocyst is needed to identify those with the highest implantation potential. Since its inception, the blastocyst grading system developed by Gardner and Schoolcraft (1999), based on the degree of blastocyst expansion and the morphology of the inner cell mass and trophectoderm cells, was widely used in various institutions around the world. Subsequently, the association between these morphological parameters of the blastocyst and pregnancy rates has been evaluated in many studies (Gardner et al., 2000; Hill et al., 2013; Matsuura et al., 2010). The morphological evaluation of blastocysts, however, is inherently subjective, greatly observer-dependent, and the debate on which morphological parameters have the highest prognostic value is still inconclusive. Therefore, existing blastocyst grading systems need to be further improved by incorporating more objective and reproducible variables.

The developmental speed of the embryo is an important variable to consider while ascertaining its quality. For cleavage-stage embryos, it has been reported that the implantation rate is higher for embryos that cleave earlier (Lee et al., 2012). Similarly, studies involving blastocyst transfer procedures have examined differences in implantation rates between blastocysts developing up until day 5 compared with those developing up until day 6 (Liebermann and Tucker, 2006; Stehlik et al., 2005; Sunkara et al., 2010). These studies, however, did not provide details on the degree of expansion of the transferred blastocysts, and their results varied considerably. In a previous study by our group (Okimura et al., 2009), it was observed that the greater the degree of expansion of the blastocyst, the higher its implantation potential. Therefore, by examining those blastocysts with a uniform degree of expansion, the association between embryo developmental speed to blastocyst and the success rates may be examined more accurately. The aim of the present study was to analyse success rates after single vitrified-warmed blastocyst transfer (SVBT) according to women's age and embryo developmental speed, and to establish a novel blastocyst grading system that could help to predict outcome after SVBT.

Materials and methods

Patients and study design

This retrospective study was reviewed and approved by the independent Institutional Review Board of Kato Ladies Clinic, Tokyo (IRB approval number: 13–20, approved 12 September 2013). Written informed consent was also obtained from all patients undergoing IVF treatment at our centre, and the signed document also informed patients that de-indentified data could be used for retrospective analysis. A total of 5948 patients undergoing 7341 SVBT cycles who fulfilled the following inclusion criteria were included in this retrospective analysis: women's age 45 years or less; women undergoing SVBT with a 175–184 μm sized blastocyst showing a uniform degree of expansion; and availability of follow-up data on pregnancy outcome. Cycles resulting in monozygotic twin pregnancies and ectopic pregnancies were excluded. At our centre, the blastocyst transfer strategy was routinely indicated in cases of tubal factor infertility (e.g. tubal obstruction, hydrosalpinx or a history of extrauterine pregnancy), and after previous failed cycles with single cleavage-stage embryo transfers, and constituted a proportion of treatments carried out in our centre (Kato et al., 2012).

In the first part of the analysis, clinical pregnancy rates (CPR) were analysed in five subgroups that were divided before vitrification, according to five women's age (≤34, 35–37, 38–39, 40–41, and 42–45 years) and five blastocyst growth rate categories (≤119, 120–129, 130–139, 140–149, and ≥150 h). A simple grading system (grades A to E corresponded to a CPR of ≥54, 44–54, 34–43, 24–34 and <24%, respectively) was established based on actual clinical pregnancy rates observed in the first half of the cohort (6046 cycles carried out between 2006 and 2011). Subsequently, the predictive value of the above grading system was evaluated in the second half of the cohort (1295 cycles carried out during 2012). Additionally, ongoing pregnancy and delivery rates for grade were also calculated in each cohort.

Minimal ovarian stimulation, oocyte retrieval and fertilization procedures

All patients underwent a clomiphene-based minimal ovarian stimulation protocol or drug-free natural cycle IVF treatment (Kato et al., 2012; Teramoto and Kato, 2007). Oocyte retrieval was conducted without any anaesthesia, using a fine 21-22 G needle (Kitazato, Japan). Follicular flushing was not used during oocyte retrieval. Conventional insemination was carried out about 3 h after retrieval, ICSI was carried out 5 h after retrieval. P1/cleavage stage medium (Irvine Scientific, USA) or human tubal fluid (HTF; Irvine Scientific, USA) with 10% serum substitute supplement (SSS; Irvine Scientific, USA) was used as the culture medium after insemination.

Embryo culture, blastocyst monitoring and vitrification

Fertilization assessment was carried out 16–20 h after insemination. Normally, fertilized zygotes with two pronuclei were cultured individually in a drop of 20 μL of Quinn's Advantage Protein Plus cleavage medium (SAGE, USA) from days 1–3. The embryos were transferred to Quinn's Advantage Protein Plus blastocyst medium (SAGE, USA) on day 3 and cultured until day 5 to 7. All embryos were cultured at 37°C under the gas phase of 5% O2, 5% CO2 and 90% N2, with 100% humidity in water jacket small multigas incubators or dry desktop incubators (Astec, Japan).

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Only patients having a blastocyst with an inner diameter between 175 and 184 μm with a uniform degree of blastocoel expansion were included in the present study. As per centre protocol, development of days 5 to 7 embryos were closely monitored and checked routinely two to four times daily. Observations usually took place at 8am and, if the embryo had already reached the blastocyst stage, its size was checked every 3 h until 5pm. If the embryo had not yet reached the blastocyst stage, it was checked again 6 h later at 2pm and 3 h afterwards at 5pm. If the embryos still had not reached blastocyst, with an inner diameter of over 160 μm or hatching blastocyst, it was checked again the next day at 8am. Blastocyst checking was only carried out by well-trained senior embryologists, and each observation was completed within 1 min. An inverted microscope (IX-71, Olympus, Japan or TE2000-U, Nikon, Japan) and corresponding imaging software (CellSens; Olympus, Japan; or OCTAX EyeWare™; OCTAX Microscience GmbH, Germany) were used to measure the blastocyst's inner diameter.

Those blastocysts reaching an inner diameter of over 160 μm or hatching blastocyst by day 5 to 6 were vitrified immediately according to the Cryotop method (Kuwayama, 2007). If the developing embryo did not fulfill the desired criteria, it was cultured further up until a maximum of day 7. For day 7 blastocysts, vitrification criteria were blastocysts reaching an inner diameter of over 180 μm or hatching. If the embryo did not fulfill the above criteria by day 7 it was discarded. The time interval from insemination to blastocyst vitrification was measured in hours (rounded up or down at a 0.5 h interval) to quantify the embryo developmental speed until blastocyst stage.

Post-warming embryo culture, embryo transfer procedure and outcome measures

During the study period, only single-embryo transfers were carried out in our centre, and an exclusive single-embryo transfer policy was strictly observed. Therefore, the cohort was analysed on a per cycle basis. On day 4.5 to 5 after ovulation, SVBT were transferred during a spontaneous natural cycle, as mentioned previously (Kato et al., 2012). After warming, surviving blastocysts were cultured for 30 mins to 2 h until blastocoel re-expansion was confirmed. Only those blastocysts were transferred in which the blastocoel size remained the same or increased compared with before the vitrification state. Degenerating blastocysts were discarded.

The embryo transfer procedure was conducted under vaginal ultrasonography guidance using a specially designed soft silicone inner catheter (Kitazato, Japan) by placing a single blastocyst, suspended in minimal medium volume, in the upper part of the uterine cavity. Dydrogesterone (30 mg/day orally, Daiichi-Sankyo, Japan) was routinely administered during the early luteal phase after the transfer procedure. Moreover, intramuscular (125 mg/5 days, Fuji Pharma, Japan) or intravaginal (25 or 50 mg/day, preparation in-house) progesterone was also administered until the week 9 of pregnancy in cases in which endogenous progesterone production from the placenta was found to be insufficient. During the first trimester, pregnancies were followed weekly by hormonal measurements, and ultrasound until about 9 weeks of ongoing gestation, at which point patients were referred to their treating obstetrician for subsequent care.

Main outcome measures were clinical pregnancy rate (with a confirmed gestational sac at 6–7 weeks of pregnancy), ongoing pregnancy rate (a confirmed fetal heart beat at 9 weeks of pregnancy) and delivery rate (live birth at 22 weeks of pregnancy over) per embryo transfer procedure. Pregnancy outcomes were ascertained by a written patient questionnaire, by the treating obstetrician, or both. Nominal variables were analysed by the Fisher's exact test or the Cochran–Armitage test for trend as appropriate. $P < 0.05$ was considered statistically significant.

Results

Observed clinical pregnancy rates after SVBT in the 2006–2011 cohort stratified by women's age and blastocyst growth rate are presented in **Table 1**. Grades were established

Table 1 Clinical pregnancy rates of the 2006–2011 cohort in subgroups stratified by women's age and time required for blastocyst expansion after insemination.

Age (years)	*Time required for blastocyst expansion after insemination (h)*					P[a]
	<120 n *(%)*	*120–129* n *(%)*	*130–139* n *(%)*	*140–149* n *(%)*	*>149* n *(%)*	
<35	240/363 (66.1) A	147/228 (64.5) A	72/137 (52.6) B	127/347 (36.6) C	6/17 (35.3) C	<0.0001
35–37	249/407 (61.2) A	151/254 (50.4) A	74/155 (47.7) B	181/474 (38.2) C	7/20 (35.0) C	<0.0001
38–39	178/320 (55.6) A	130/228 (57.0) A	61/148 (41.2) C	136/413 (32.9) D	5/19 (26.3) D	<0.0001
40–41	145/306 (47.7) B	101/222 (45.5) B	44/122 (36.1) C	123/482 (25.5) D	3/28 (10.7) E	<0.0001
42–45	112/313 (35.8) C	62/236 (26.3) D	25/160 (15.6) E	105/626 (16.8) E	1/21 (4.8) E	<0.0001

In each cell, the upper portion indicates the observed clinical pregnancy rate and the lower portion indicates the attributed grade (grades A to E correspond to a clinical pregnancy rate of ≥54, 44–54, 34–43, 24–34 and <24%, respectively).
[a]Cochrane–Armitage test for trend.

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arbitrarily in 10% intervals corresponding to a CPR of ≥54, 44–53.9, 34–43.9, 24–33.9 and less than 24% for grades A to E, respectively. In a similar way, the ongoing pregnancy and delivery rates in the same 2006–2011 cohort are presented in Table 2. For all outcome variables, pregnancy rates were significantly different ($P < 0.0001$) between groups and decreased progressively with increasing women's age and increased time needed for blastocyst expansion. Observed CPR, ongoing pregnancy rate and delivery rates in the 2006–2011 cohort stratified by grades A to E are shown in Table 3. The CPR, ongoing pregnancy rate and delivery rate differed significantly among the grades ($P < 0.05$ for all variables). A comparison of clinical pregnancy rates stratified by grades A to E in the 2006–2011 and 2012 cohort is presented in Table 4. No significant differences were observed in these outcomes between subgroups, and the two cohorts confirmed a good predictive value of the novel grading system.

Discussion

The present study showed that clinical pregnancy rates after vitrified blastocyst transfer could be accurately predicted using objective parameters only, such as women's age and embryo developmental speed. This implies that more complicated morphological grading variables are not an obligatory part of a valid blastocyst grading system. This new grading system developed in the 2006–2011 cohort showed good association with outcomes observed in the 2012 cohort. Furthermore, the novel grading system might also be useful in predicting more robust outcomes, such as ongoing pregnancy and delivery rates.

Previous studies have compared pregnancy rates according to the number of days required to reach the blastocyst stage (Liebermann and Tucker, 2006; Stehlik et al., 2005; Sunkara et al., 2010). In our unique cohort of SVBT, however, the association between embryo developmental speed and implantation potential could be analysed even more accurately, because all the patients invariably underwent single blastocyst transfer (eliminating multiple embryo transfer as a confounder), a standard SVBT protocol was used (embryo transfer at day 4.5–5 after ovulation) and the degree of blastocyst expansion was similar for all included cycles.

The speed at which the embryo developed to the blastocyst stage seemed to be a highly important parameter in our novel blastocyst grading system. Morphological evaluation of blastocysts may yield ambiguous and varying results, depending on the institutions and embryologists involved. It is inherently a more subjective process, especially when compared with the simpler and more standardized morphological evaluation criteria used for cleavage-stage embryos. In contrast, our new grading system could yield more accurate results of blastocyst evaluation because it is based on objective parameters, whereby subjective variations during its application can be largely avoided. In the present study, the relationship between the morphology of the blastocyst inner cell mass/trophectoderm and the blastocyst growth rate (mean ± SD) in the cohort 2006–2011 was as follows: grade AA: 118.2 ± 5.2 h; grades AB–BB: 119.5 ± 6.5 h and grades BC–CC: 134.1 ± 12.1 h. This suggests that blastocysts showing adequate morphological characteristics might also have a higher developmental speed. This implies that a simple evaluation based on

Table 2 Ongoing pregnancy and delivery rates of the 2006–2011 cohort in subgroups stratified by women's age and time required for blastocyst expansion after insemination.

	Age (years)	Time required for blastocyst expansion after insemination (h)								P[a]
		<120		120–129		130–139		140–149		>149		
		n	%	n	%	n	%	n	%	n	%	
Ongoing pregnancy rate	<35	228/363	62.8	141/228	61.8	68/137	49.6	114/347	32.9	6/17	35.3	<0.0001
	35–37	224/407	55.0	138/254	54.3	65/155	41.9	156/474	32.9	6/20	30.1	<0.0001
	38–39	162/320	50.6	115/228	50.4	48/148	32.4	103/413	24.9	2/19	10.5	<0.0001
	40–41	125/306	40.8	85/222	38.3	34/122	27.9	100/482	20.7	2/28	7.1	<0.0001
	42–45	83/313	26.5	38/236	16.1	20/160	12.5	74/626	11.8	1/21	4.8	<0.0001
Delivery rate	<35	208/363	57.3	115/228	50.4	59/137	43.1	96/347	27.7	6/17	35.3	<0.0001
	35–37	182/407	44.7	123/254	48.4	47/155	30.3	130/474	27.4	4/20	20.0	<0.0001
	38–39	129/320	40.3	96/228	42.1	35/148	23.6	79/413	19.1	2/19	10.5	<0.0001
	40–41	85/306	27.8	56/222	25.2	22/122	18.0	73/482	15.1	2/28	7.1	<0.0001
	42–45	52/313	16.6	26/236	11.0	14/160	8.8	49/626	7.8	0/21	0.0	<0.0001

[a]Cochrane-Armitage test for trend.

Table 3 Clinical pregnancy rate, ongoing pregnancy rate and delivery rates stratified by grades A to E in the 2006–2011 cohort.

Grades	*A*	*B*	*C*	*D*	*E*
Vitrified blastocyst transfers	1800	820	1441	1150	835
Clinical pregnancy rate	1095 (60.8%)	392 (47.8)	538 (37.3)	326 (28.3)	134 (16.0)
Ongoing pregnancy rate	1008 (56.0)	343 (41.8)	447 (31.0)	243 (21.1)	97 (11.6)
Delivery rate	853 (47.4)	247 (30.1)	345 (23.9)	180 (15.7)	65 (7.8)

A significant difference was found in the rates among the grades ($P < 0.05$).

Table 4 Comparison of clinical pregnancy rates according to grades A to E in the 2006–2011 and 2012 cohorts.

Grade	*2006–2011*		*2012*	
	n	%	n	%
A	1095/1800	60.8	251/387	64.9
B	392/820	47.8	97/192	50.5
C	538/1441	37.3	104/305	34.1
D	326/1150	28.3	69/225	30.7
E	134/835	16.0	24/186	12.9

No significant between-group differences were found.

objective developmental speed may provide similar results to an accurate but more time-consuming morphological evaluation. This study, however, did not compare timing of blastocyst expansion with blastocyst morphology as predictors of pregnancy outcome. Therefore, further studies are required to analyse the correlation between our novel evaluation methods and blastocyst morphology.

In the present study, cycles were also evaluated based on the woman's age. It has been already reported that the pregnancy rate using blastocyst transfer reduces progressively with increasing patient age (Goto et al., 2011). In a previous retrospective study, low blastocyst formation rate was also observed in the advanced age group (Kato et al., 2012). According to guidelines from the Japan Society of Obstetrics and Gynecology (2006), donor eggs cannot be used for IVF even for advanced maternal aged patients in Japan, which underlines the importance of considering women's age while evaluating blastocysts.

Our grading system was developed in a large cohort where all patients underwent minimal ovarian stimulation or natural cycle IVF. In most cases of minimal ovarian stimulation, and especially in natural cycle IVF treatment, only a single (if any) blastocyst is available for embryo transfer. Therefore, few opportunities are available for blastocyst selection for transfer. This novel grading system, however, might also be useful in the different setting of conventional ovarian stimulation where multiple embryos could be selected. Moreover, while designing this novel, more objective blastocyst grading system, we also attempted to ensure efficient prediction and simplicity at the same time, whereby patients could easily understand the proposed grading. Similar to Gardner's classification (Gardner and Schoolcraft, 1999), our grading system can be easily understood by patients; thus, the unrealistic expectations of older patients can be prevented and worry and concern among younger patients can be mitigated. Additionally, an even more accurate prediction of the success rates might be possible by combining this grading system with the classical Gardner's classification.

The time of sperm penetration into the oocyte may vary depending on the fertilization method used. A limitation of the current study is that we did not evaluate any effect of the insemination method used (conventional IVF compared with ICSI). Similarly embryo developmental speed was measured by using multiple daily observations at discrete time points, avoiding the use of expensive time-lapse technology. In future studies, data from presumably more precise time-lapse observations could be incorporated to enhance the accuracy of our proposed blastocyst grading system.

Conclusion

A novel blastocyst grading system was established using women's age and blastocyst growth rate as objective parameters. This blastocyst grading system might be superior to previous grading systems because it is easy to implement and it could avoid institution- and observer-related variations. Although a retrospective validation suggested a good predictive value of the novel grading system, it should be evaluated in a future, prospective study.

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Dr Keiichi Kato and Dr Satoshi Ueno contributed equally to writing this paper, and should be considered joint authors. The authors wish to thank Daniel Bodri MD. MSc. PhD. (Palma de Mallorca, Spain) for his help in editing the initial manuscript draft.

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