Blastocyst Quality Scoring Based on Morphological Grading Correlates with Cell Number

Koji MATSUURA[1] PhD, Nobuyoshi HAYASHI[2] MD PhD, Chisato TAKIUE[2] BS,

Rei HIRATA[2] BS, Toshihiro HABARA[2] MD PhD, Keiji NARUSE[1,*] MD PhD

[1]*Cardiovascular Physiology, Graduate School of Medicine, Dentistry and*

Pharmaceutical Sciences, Okayama University, Okayama, Japan

[2]*Okayama Couples Clinic, Okayama, Japan*

Running head: Correlation between BQS and cell number

*Corresponding author

E-mail address: knaruse@md.okayama-u.ac.jp

Fax: +81-86-235-7430

Abstract

Blastocyst quality score (BQS), first reported by Rehman et al., is a numerical blastocyst-morphology grading system based on the criteria established by Gardner and Schoolcraft. We demonstrate a positive correlation between the calculated BQS score and cell number by staining thawed human embryos and suggest that BQS can be applied to evaluate culture systems clinically.

An investigation of embryo quality before transplantation is an important process in the light of recent recommendations for single-embryo transfers (SETs) in the US, EU, and Japan (1, 2) and/or evaluation of improved embryo culture systems. Blastocyst morphology has been clinically assessed during the selection of blastocysts for transfer and cryopreservation, and the morphological grading scheme has gained wide acceptance (3). It would be sufficient to evaluate the quality of several embryos after *in vitro* culture using a non-numerical morphological grading scheme. However, the disadvantages of a non-numerical index are (1) an inability to calculate the mean grade of the whole embryo cohort at any given point and (2) the difficulty in grouping embryos.

A numerical index for blastocyst quality has been reported by Rehman et al. (4, 5). Blastocyst quality score (BQS) is a numerical blastocyst morphological grading system based on the criteria established by Gardner and Schoolcraft (4-6). They discussed a possible correlation between BQS and estimated cell numbers in blastocysts. In the literature, we found descriptions of counted cell numbers in nuclear stained blastocysts on days 5 and 6. However, the relationship between morphological grading and cell numbers in blastocysts was speculative. We consider that such studies were reasonable. However, they did not stain blastocysts, count cell numbers in the blastocysts, and grade blastocyst quality based on morphology. Therefore, they did not

demonstrate the relationship between BQS based on Gardner's criteria and cell numbers in blastocysts.

If the relationship between morphological grading and cell numbers in blastocyst could be demonstrated experimentally, BQS would be widely accepted and applied as a numerical index to rate blastocyst quality. Because it is clinically difficult to confirm the correlation between BQS and cell number, a study of thawed human embryos is more suitable. In this report, we cultured embryos from frozen human 3–11-cell embryos for 48 h and investigated the correlation between BQS and cell numbers in the blastocyst. The results suggest that this numerical index can be applied to assess embryo culture conditions clinically.

We used 3–11-cell stage embryos frozen by the slow method 3 days after collection from May 2000 to December 2004 (7), and extended the prospective study including 220 fertilized human embryos that would have been discarded with consensus after pregnancy. This study was approved by the Ethics Committee of Okayama University Graduate School of Medicine. The frozen embryos were thawed with THAW-KIT1^{TM} (Vitrolife, Göteborg, Sweden), and the viability of the thawed embryos was approximately 80%. Each human thawed embryo was cultured in a 20 μL microdrop of Global$^{®}$medium (LifeGlobal, Canada) covered with mineral-oil for 48 h in a 50 L multi-gas incubator (ASTEC, Japan).

Gardner blastocyst grades were converted into BQS as previously reported (4).

We scored expansion rate depending on the developmental stage, and graded according to quality using published criteria with slight modifications (6, 8). Early blastocysts (scored as 1–2) were assessed based on morphological appearance as follows: A (many equal-shaped cells), B (many unequal-shaped cells), and C (few cells and degeneration) (8). For 2A embryos, the multiplicative blastocyst quality score (BQS = R[expansion] × R[morphology]) was equal to 2 × 3, giving a BQS score of 6. Both ICM and TE grades were defined when the expansion rate was 3 or 4. For 3AB embryos, the multiplicative blastocyst quality score (BQS = R[expansion] × R[ICM] × R[TE]) was equal to 3 × 3 × 2, giving a BQS score of 18.

The human blastocyst cells were stained with Hochest 33342, observed by confocal microscopy (FV-1000 Olympus Japan), and a 3D image was constructed (9). It was difficult to count ICM and TE cells individually, because the cell borders were unclear following nuclear staining with Hochest 33342 due to treatment of the blastocysts for the fluorescence observation. We used Pearson's product–moment correlation coefficients (10), and a P value < 0.001 was considered significant.

We investigated Gardner blastocyst grade and cell number for approximately 80 blastocysts. Figure 1A shows the Gardner Scoring system versus a scatter plot of the actual cell numbers of blastocysts, suggesting that the positive correlation. The statistical analysis is difficult and laborious, because the grading (1C − 4AA) is not numerical. Figure 1B also shows the positive correlation between the BQS results and

cell number in the blastocysts. In general, the correlation efficient (r) is more relevant in a log–log plot than in a linear plot, so we set the logarithm of the cell number against the cell division number. The BQS logarithm, in which the expansion rate is over 3, was the summation of all 3 parameters [equation 1].

$$\log_2 BQS = \log_2 R[\text{expansion}] + \log_2 R[\text{ICM}] + \log_2 R[\text{TE}] \qquad [1]$$

Our results suggest that there is an increased total cell number in morphologically improved ICM and TE with an expanded blastocyst, and that the BQS logarithm is a non-invasive index of cell division progression. This is consistent with the discussion in the report by Bukulmez et al. (4). We demonstrated that blastocyst cell number can be roughly evaluated from morphology

BQS can be applied to estimate cell numbers by a noninvasive assessment, and can be a criteria to rate high grade embryos between several groups. Whole samples are usually separated into groups to evaluate new medium and embryo culture protocols in prospective and retrospective studies. When a non-numerical index such as conventional embryo grading system is used, we need additional repetitions to judge protocol effectiveness, due to the small numbers of embryo quality levels and the requirement for a large number of samples to produce statistical significance. However, blastocyst quality numerical index without staining can be applied for such statistical analyses. Thus, we suggest that BQS could be applied clinically to evaluate culture system media or environment.

We attempted to apply this numerical index to investigate the effectiveness of the recently developed tilting embryo culture system (TECS) by comparing BQSs of developed blastocysts in static control groups during thawed embryo development (11). TECS is an electric device used to construct a dynamic culture system, which uses a mechanical stimulus that can easily be adapted to conventional static culture platforms. Previously, we found that human thawed embryos showed slight developmental improvement to the blastocyst stage following TECS culture compared to static culture groups. The mean cell number of developed blastocysts of Day 5 by the TECS was 43±3 cells (n=24, ±SEM), while that of the static culture group was 34±3 cells (n=18, ±SEM) (11). There was significant difference in the averages of the cell numbers between the two groups ($P<0.05$). These results suggest that TECS increased the number of cells in the human blastocyst, and that TECS could enhance cell division of human embryos. However, blastocyst quality in a conventional static culture group should be compared with that in a TECS culture group to apply this device to clinical routines. BQS is a numerical index to evaluate the culture system, and most ART laboratories adopt Gardner's criteria and the grading can be transferred readily to BQS. We suggest these evaluation schemes, including BQS, not only for this apparatus but also for other culture systems and culture media in clinical studies.

In conclusion, we demonstrated a positive correlation between BQS and estimated cell numbers in blastocysts, and that BQS can be clinically useful to evaluate blastocyst quality and culture systems.

REFERENCES:

(1) Gerris J, De Neubourg D, Mangelschots K, Van Royen E, Van de Meerssche M, Valkenburg M. Prevention of twin pregnancy after in-vitro fertilization or intracytoplasmic sperm injection based on strict embryo criteria: a prospective randomized clinical trial. Hum Reprod 1999; 14:2581–7.

(2) Van Royen E, Mangelschots K, De Neubourg D, Valkenburg M, Van de Meerssche M, Ryckaert G *et al.* Characterization of a top quality embryo, a step towards single-embryo transfer. Hum Reprod 1999; 14:2345–9.

(3) Gardner DK, Lane M, Stevens J, Schlenker T, Schoolcraft WB. Blastocyst score affects implantation and pregnancy outcome. Fertil Steril 2000; 73:1155-8.

(4) Bukulmez O, Carr BR, Doody KM, Doody KJ. Serum cetrorelix concentrations do not affect clinical pregnancy outcome in assisted reproduction. Fertil Steril 2008; 89:74-83.

(5) Rehman KS, Bukulmez O, Langley M, Carr BR, Nackley AC, Doody KM. Late stages of embryo progression are a much better predictor of clinical pregnancy than early cleavage in intracytoplasmic sperm injection and in vitro fertilization cycles with blastocyst-stage transfer. Fertil Steril 2007; 87:1041-52.

(6) Gardner DK, Schoolcraft WB. In vitro culture of human blastocysts. Jansen R, Mortimer D, ed. Towards reproductive certainty: fertility and genetics beyond 1999: the plenary proceedings of the 11th World Congress on In Vitro Fertilization &

Human Reproductive Genetics. Pearl River, NY: Parthenon; 1999: 378-88.

(7) Cohen J, Simons RF, Edwards RG, Fehilly GB, Fishel SB. Pregnancies following the frozen storage of expanding human blastocysts. Journal of In Vitro Fertilization and Embryo Transfer 1985; 2:59-64.

(8) Mukaida T, Nakamura S, Tomiyama T, Wada S, Oka C, Kasai M, *et al.* Vitrification of human blastocysts using cryoloops: clinical outcome of 223 cycles. Human. Reprod 2003; 18:384-91.

(9) Hardy K, Handyside AH Winston RML. The human blastocyst: cell number, death and allocation during late preimplantation development in vitro. Development 1989; 107:597-604.

(10)   Wilding M, Dale B, Marino M, di Matteo L, Alviggi C, Pisaturo ML, *et al.* Mitochondrial aggregation patterns and activity in human oocytes and preimplantation embryos. Hum Reprod 2001; 16:909-17.

(11)   Matsuura K, Hayashi N, Kuroda Y, Takiue C, Hirata R, Takenami M *et al.* 2009 Improved Development of Mouse and Human Embryos by Tilting Embryo Culture System. RBM Online in press.

FIGURE CAPTION (Figure 1)

A: The Gardner Scoring versus a scatter plot of the actual cell numbers of embryos. Numbers in circles are expansion rate. Morphology rates (A, B, and C) are outside of

the horizontal axis. B: Correlation of $\log_2$ (BQS) and $\log_{10}$ of the blastocyst cell number

($P < 0.0001$).