Reproductive BioMedicine Online (2015) **31**, 523–530

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ARTICLE

A clinically useful simplified blastocyst grading system

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Abstract The aim of this study was to investigate whether a new simplified blastocyst grading system (A: fully expanded, clear inner cell mass, cohesive trophectoderm; B: not yet expanded, clear inner cell mass, cohesive trophectoderm; C: small inner cell mass ± irregular trophectoderm ± excluded/degenerate cells) was clinically useful. All day-5 single embryo transfers between 15 June 2009 and 29 June 2012 were reviewed. Implantation, clinical pregnancy and live birth rates were related to embryo quality. Five embryologists were asked to grade and decide the clinical fate of 80 images of day-5 embryos on two occasions 4–6 weeks apart. Implantation, clinical pregnancy and live birth rates decreased with deteriorating embryo quality. A highly significant ($P < 0.01$) difference was observed between the groups. Inter-observer agreement was substantial for grade allocation (K = 0.63) and clinical decision-making (K = 0.66). Intra-observer agreement ranged from substantial (K = 0.71) to almost perfect (K = 0.88) for grade allocation, and was almost perfect for clinical fate determination (K ≥ 0.84). This grading system is quick and easy to use, effectively predicts IVF outcome and has levels of agreement similar to, if not better than, those associated with more complex grading systems.

KEYWORDS: blastocyst, grading, variability

Introduction

Blastocyst culture increases the success rate of assisted reproduction techniques because it permits better embryo selection after genomic activation, is associated with better endometrial receptivity, or both (Hardarson et al., 2012). Extended culture to the blastocyst stage also enables selection of the most viable embryo from a cohort, thus reducing

http://dx.doi.org/10.1016/j.rbmo.2015.06.017
1472-6483/

the need to transfer multiple embryos to achieve reasonable success rates with a consequent reduction in the incidence of multiple pregnancy (Hardarson et al., 2012).

Time-lapse microscopy, molecular karyotyping and proteomics and metabolomics are increasingly being used in many units to aid identification of the optimum embryo; however, the decision of which blastocyst to transfer is still largely made on the basis of morphological assessments conducted in the IVF laboratory at the time of embryo transfer (Dokras et al., 1993; Gardner et al., 2004). Multiple blastocyst grading systems are in existence to aid this process. Regardless of the grading system used, multiple studies have demonstrated a strong correlation between blastocyst quality and implantation and clinical pregnancy rates (Balaban et al., 2000, 2006; Gardner et al., 2000, 2004).

Most grading systems currently used for assessing the viability of IVF embryos are subjective, relying on visual inspection of morphological characteristics of the embryos that are qualitatively evaluated. Grading based on qualitative criteria is imprecise and inevitably results in inter-observer, and to some extent intra-observer, variability. Gardner et al.'s (1998) grading system, which is used by many clinics, allows for 54 different permutations and hence considerable scope exists for different embryologists to allocate the same blastocyst a different grade (inter-observer variation) or the same embryologist to allocate the same blastocyst a different grade if assessed on a different occasion (intra-observer variation).

Clinically, it is important to try to minimize variability in embryo scoring because the grade of the embryo is used to predict the likelihood of successful treatment, and therefore influences the decision on which embryo to replace and how many embryos to transfer. It also dictates how couples undergoing IVF treatment are counselled about the likelihood of implantation, clinical pregnancy, multiple pregnancy and live birth so that their expectations can be appropriately managed.

It has been suggested that minimizing inter- and intra-observer variability could be achieved by having all the embryo grading done by a single observer or by having multiple embryologists evaluate each embryo and then decide upon the grade by consensus (Baxter Bendus et al., 2006). Neither of these options are practical for most IVF clinics.

Simplifying the grading system will, by definition, reduce variability, as has been shown (Al-Aynati et al., 2003). In 2009, Dr Cecilia Sjoblom devised a simplified blastocyst grading system (**Table 1**) based on our unit's prior experience of the Gardner system. An essential component of any grading system is that it is not only accurate and reproducible but that it can be used to predict outcome. We therefore investigated our simplified blastocyst grading system to determine whether or not it could be used to predict clinical outcome in terms of implantation, clinical pregnancy and live birth and is consistent and accurate with minimal inter- and intra-observer variability.

Materials and methods

In 2009, when we decided to simplify our blastocyst grading system, it was noted from our experience of using the Gardner grading system that any blastocyst that had a Gardner expansion grade of 4, 5 or 6 (i.e. fully expanded) with an inner cell mass and trophectoderm grade 'A' or 'B' had the highest implantation rate, so this became the criteria for the grade 'A' blastocysts in our simplified system. Any blastocyst that was not fully expanded but had an inner cell mass and trophectoderm grade 'A' or 'B' had a slightly lower implantation rate, and this became the benchmark for our grade 'B' blastocysts. Any blastocyst that had a Gardner grade 'C' for inner cell mass or trophectoderm, regardless of expansion status, had a much lower implantation rate and this became our grade 'C' blastocyst (**Table 1**).

Determination of prognostic potential

We reviewed all single (elective or otherwise), fresh or frozen day-5 embryo transfers at Nurture Fertility between 15 June 2009 and 29 June 2012. All participants had undergone IVF–ICSI treatment using a standard long agonist or antagonist protocol, depending on ovarian reserve tests as previously described (Jayaprakasan et al., 2010, 2014).

The following data were collected: age, ethnicity, smoking status, BMI, type of treatment (IVF–ICSI) and grade of blastocyst transferred (as judged by the duty embryologist on the day of embryo transfer). Implantation (defined as a positive urinary pregnancy test performed 18 days after oocyte retrieval), clinical pregnancy (defined as ultrasonographic evidence of at least one fetal heartbeat) and live birth (defined as delivery of a live baby at more than 24 weeks gestation) rates were recorded. Information was obtained from IVF unit records. Embryos that were assigned ambiguous grades (for example A/B, B/C) not compliant with the grading system described above were excluded, as were any transfers where outcome data were not available.

Determination of inter- and intra-observer variability

Five embryologists were asked to view 80 still images of day-5 embryos. All embryologists had a life sciences degree and were State Registered Clinical Scientists with the Health and Care Professions Council. They had been qualified for between 7 and 20 years, and had between 2 and 5 years' experience using this simplified blastocyst grading system.

Still images were obtained by one of the authors (SB). The images were randomly grouped into subsets comprising five embryos. Participants were asked to grade the embryos using the simplified blastocyst grading system described above. They were also asked, out of each subset of five images, which embryo they would preferentially transfer if presented with that cohort, which, if any, they would freeze (grade A or B blastocysts only as per unit policy) and which, if any, they would discard. They were blinded to the assessments of the other embryologists to minimize bias.

The same cohort of embryologists were asked to grade the same set of still images 4–6 weeks after the initial assessment. The order of the still images had been randomly manipulated before the second assessment in an attempt to eliminate recall bias.

According to the Medical Research Council Health Research Authority, ethical approval was not required for this