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Original Research Article

Assessment of human embryos by time-lapse videography: A comparison of quantitative and qualitative measures between two independent laboratories

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ABSTRACT

A total of 488 Day 3 human embryos with known implantation data from two independent *in vitro* fertilization laboratories were included for analysis, with 270 from Fertility North (FN) and 218 from Canberra Fertility Centre (CFC). Implanting embryos grew at different rates between FN and CFC as indicated in hours of the time intervals between pronuclear fading and the 4- (13.9 ± 1.1 *vs.* 14.9 ± 1.8), 5- (25.7 ± 1.9 *vs.* 28.4 ± 3.7) and 8-cell stages (29.0 ± 3.2 *vs.* 32.2 ± 4.6), as well as the durations of 2- (10.8 ± 0.8 *vs.* 11.6 ± 1.1), 3- (0.4 ± 0.5 *vs.* 0.9 ± 1.2), and 4-cell stages (11.8 ± 1.4 *vs.* 13.6 ± 2.9), all $p < 0.05$. The application of a previously published time-lapse algorithm on ICSI embryos from the two participating laboratories failed to reproduce a predictive pattern of implantation outcomes (FN: AUC = 0.565, $p = 0.250$; CFC: AUC = 0.614, $p = 0.224$). However, for the qualitative measures including poor conventional morphology, direct cleavage, reverse cleavage and <6 intercellular contact points at the end of the 4-cell stage, there were similar proportions of embryos showing at least one of these biological events in either implanting (3.1% *vs.* 3.3%, $p > 0.05$) or non-implanting embryos (30.4% *vs.* 38.3%, $p > 0.05$) between FN and CFC. Furthermore, implanting embryos favored lower proportions of the above biological events compared to the non-implanting ones in both laboratories (both $p < 0.01$). To conclude, human embryo morphokinetics may vary between laboratories, therefore time-lapse algorithms emphasizing quantitative timing parameters may have reduced inter-laboratory transferability; qualitative measures are independent of cell division timings, with potentially improved inter-laboratory reproducibility.

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1. Introduction

Time-lapse videography permits continuous monitoring of human embryo growth over the entire culture period to assess both quantitative morphokinetic measures [1–7] and the occurrence of qualitative indicators of atypical cleavage [7–11]. The use of enclosed incubators equipped with built-in video cameras does not appear to be detrimental to embryo quality [12], possibly improving culture conditions [13], and may be advantageous in improving clinical pregnancy rates [4,14] although further high quality evidence is required before routine application [15,16]. Selection of the best embryo(s) for transfer using time-lapse videography requires a set of defined limits which embryos can be assessed against, and published algorithms that exist use predominantly morphokinetic parameters [6,17,18]. Unfortunately, such algorithms may lose discriminatory power for embryo selection when transferred for use in other laboratories [19–21]. This has led to the exploration of algorithms based upon other markers, such as qualitative measures of cleavage [8].

The reason behind the limited transferability of algorithms is unclear. However, if one applies the same criteria to the parameters measured by time-lapse videography as those applied to any other diagnostic test, then each parameter measured should have minimal technical and biological variability [22]. Previously, an assessment of technical precision in time-lapse observations found whilst measurements were more objective than conventional assessments, some parameters measured were more variable than others [23]. It must also be noted that the growth of embryos is not constant, and may be influenced by external factors. Such confounding factors have been associated with the patient (*e.g.*, the stimulation regimen used for ovarian stimulation [24,25], gonadotropin dose and hormonal levels [24,26], smoking [27], the presence of hyperandrogenic polycystic ovarian syndrome in the female [28], and sperm DNA fragmentation in the male [29]) and also the culture system (*e.g.*, oxygen concentration in the incubator [30] and the use of different culture media formulations [31]).

The successful application of a time-lapse algorithm to assess embryo implantation potential in different laboratories requires that the embryos must behave in a similar manner between laboratories despite differences in laboratory culture conditions and patient profiles. The aim of the present study was therefore to describe the culture system and cycle data for two independent laboratories, and then compare the time-lapse videography findings for (a) the quantitative morphokinetic parameters of embryos with known implantation data (KID), (b) the suitability of a published algorithm to assess the implantation potential of embryos transferred, and (c) the prevalence of qualitative biological events in embryos categorized according to their KID status.

2. Materials and methods

2.1. Laboratories

The two Australian laboratories (Fertility North and Canberra Fertility Centre) were both accredited by the National Association of Testing Authorities for their biochemistry and andrology services, and the Reproductive Technology Accreditation Committee for their *in vitro* fertilization (IVF) treatment services. In addition, both laboratories participate in the embryo grading and embryo time-lapse modules of the External Quality Assurance Schemes for Reproductive Medicine (Northlands, Western Australia, Australia).

Table 1 – Comparisons of cycle characteristics between Fertility North and Canberra Fertility Centre.

	Fertility North	Canberra Fertility Centre
No. cycles included	212	160
Age (y, mean ± SD)	34.5 ± 4.6	36.3 ± 5.0*
No. IVF:ICSI cycles	84:128	89:71*
No. Agonist:antagonist cycles	89:123	61:99
Peak E_2 (pmol/L, mean ± SD)	6612.2 ± 3405.1	7766.4 ± 3918.8*
Days of FSH (mean ± SD)	10.3 ± 1.9	11.5 ± 2.0*
No. oocytes collected (mean ± SD)	9.5 ± 4.6	8.2 ± 4.1*
No. oocytes fertilized (mean ± SD)	6.6 ± 3.4	5.7 ± 2.8*
No. embryos transferred (mean ± SD)	1.3 ± 0.5	1.5 ± 0.5*

E_2 – estradiol; FSH – follicle-stimulating hormone.
* $p < 0.05$ when compared with Fertility North.

2.2. Patient recruitment and management

The study included 212 IVF or intracytoplasmic sperm injection (ICSI) treatment cycles (aged at 34.5 ± 4.6 years) at Fertility North between February 2013 and December 2014, and 160 IVF/ICSI treatment cycles (aged at 36.3 ± 5.0 years, $p < 0.05$) at Canberra Fertility Centre between January and December 2014. All cycles included KID results for transferred embryos as previously defined [8], with KID+ referring to known implanting embryos whilst KID− as known non-implanting embryos. Within these treatment cycles, a total of 488 fully annotated embryos had reached at least the 5-cell stage by 68 h post-insemination; 270 from Fertility North and 218 from Canberra Fertility Centre were included for analysis after culture in the Embryoscope™ (Vitrolife, Göteborg, Sweden) time-lapse system. Patients were managed according to the clinic's own standard operating protocols by the two respective and separate teams of clinicians. Comparisons of cycle characteristics between the two clinics are shown in Table 1.

2.3. Embryo culture

A summary of the main laboratory conditions for the two laboratories for oocyte fertilization and embryo culture is shown in Table 2. After insemination *via* either conventional IVF or ICSI, fertilized oocytes were placed in the respective Embryoscope™ incubators and cultured until at least Day 3.

2.4. Embryo assessment

Images on the Embryoscope™ were taken every 10 min for each embryo over seven focal planes. All embryos included in