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Original Research Article

Time-lapse videography of human oocytes following intracytoplasmic sperm injection: Events up to the first cleavage division

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ABSTRACT

A total of 341 fertilized and 37 unfertilized oocytes from 63 intracytoplasmic sperm injection (ICSI) treatment cycles were included for retrospective assessment using the Embryoscope™ time-lapse video system. The second polar body (pb2) extrusion occurred at 2.9 ± 0.1 h (range 0.70–10.15 h) relative to sperm injection. All oocytes reduced in size following sperm injection ($p < 0.05$) with shrinkage ceasing after 2 h in the unfertilized and at pb2 extrusion in the fertilized oocytes. Pb2 extrusion was significantly delayed for women aged >38 years compared to those <35 years (3.4 ± 0.2 vs. 2.8 ± 0.1, $p < 0.01$) or 35–38 years (3.4 ± 0.2 vs. 2.8 ± 0.1, $p < 0.01$), but timing was not related to the Day 3 morphological grades (1–4) of subsequent embryos (2.9 ± 0.1, 2.9 ± 0.1, 2.8 ± 0.2 and 3.0 ± 0.1; $p > 0.05$ respectively). A shorter time of first cleavage division relative to either sperm injection or pb2 extrusion is associated with both top grade (AUC = 0.596 or 0.601, $p = 0.006$ or 0.004) and usable embryos (AUC = 0.638 or 0.632, $p = 0.000$ respectively) on Day 3. In summary, (i) pb2 of human oocytes extrudes at various times following sperm injection, (ii) the timing of pb2 extrusion is significantly delayed when female age >38 years, but not related to subsequent embryo development, (iii) all human oocytes reduce in size following sperm injection, (iv) completion of pb2 extrusion in the fertilized oocytes is a pivotal event in terminating shrinkage of the vitellus, and (v) time to first cleavage division either from sperm injection or pb2 extrusion is a significant predictive marker for embryo quality on Day 3.

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1. Introduction

The time-course of events following sperm injection during intracytoplasmic sperm injection (ICSI) has been studied by observing individual oocytes with an inverted microscope every few hours [1,2] or by fixing oocytes at different times [3], but the use of time-lapse video [4,5] gave the first opportunity to observe oocytes continuously without interrupting the culture. Unfortunately, these earlier time-lapse studies were limited by the in-house video systems being able to video only one oocyte at a time. But technical advances have now made available time-lapse video systems that can simultaneously monitor larger numbers of oocytes and embryos [6] without compromising the culture conditions [7], enabling oocytes to be observed in greater detail after ICSI [8,9].

Morphokinetics of cell division during embryo development following ICSI were originally expressed relative to the time of sperm injection [6] and this practice continues [9]. However, recent observations on the difference in timings between in vitro fertilization (IVF) and ICSI embryos have suggested that it may be better to use a biological starting point (e.g., pronuclear fading) for timing cell divisions rather than a procedural starting point (i.e., sperm injection) to take into account differences in the rate of gamete interaction [10]. Furthermore, there is also some uncertainty on the predictive value of measurements made by time-lapse imaging relative to sperm injection to assess embryo quality, with several parameters either being significantly correlated with subsequent implantation [6] or not [11]. A re-investigation of the morphokinetics using different starting points might offer new insight into the value of time-lapse imaging to assess embryos, particularly when considering early events in the cell cycle [9], but the choice of an appropriate starting point of timing does require a full understanding of the many changes occurring within the oocyte during the fertilization process. Such changes include resumption of the second meiotic division resulting in the extrusion of the second polar body by the oocyte, and early studies have reported polar body extrusion to occur anywhere between 1 and 8 h after ICSI suggesting the maturity of the oocytes is highly variable [1,2,4,5,12]. In addition to nuclear maturation, changes in the vitellus of the oocyte occur with the volume of oocytes fertilized by ICSI being seen to reduce at 17 h post sperm injection [5].

Early event assessment up to first cleavage of human embryos has been successfully correlated with subsequent embryonic development and/or implantation results [13–15]. But a complete picture of the morphometric changes within the oocyte before and after sperm injection for oocytes undergoing ICSI, together with the variability between oocytes, is still not clear. The present study has therefore taken the opportunity to use a time-lapse video system, Embryoscope™, to (i) further describe variations in timing of the second polar body extrusion following sperm injection of oocytes, (ii) compare the size over time of fertilized and unfertilized oocytes, (iii) investigate the temporal effect of second meiotic division on the dynamic changes of oocyte size, and (iv) make a preliminary assessment of the merits of using the time to the first cleavage division relative to different biological starting points to predict embryo quality.

2. Materials and methods

2.1. Patient management and oocyte collection

Sixty-three women, aged 22–46 years, had one cycle each of ICSI between February and October 2013 with all embryos cultured in the Embryoscope™ time-lapse video incubator (Unisense Fertilitech, Aarhus, Denmark). Approval to use the Embryoscope™ was granted by the Joondalup Health Campus Research and Ethics Committee and the Reproductive Technology Council of Western Australia, and retrospective data analysis has been approved by the Human Research Ethics committee of Edith Cowan University. Informed consent was provided by all female patients and their partners.

Pituitary suppression in the women during a treatment cycle was achieved by the administration of gonadotrophin-releasing hormone (GnRH) analogs (Lucrin, Abbott Australasia, Botany, Australia; Synarel, Pfizer, West Ryde, Australia; Orgalutran, Merck Sharp and Dohme, South Granville, Australia). Ovarian stimulation was performed with recombinant follicle-stimulating hormone (rFSH) (Puregon, Schering-Plough, North Ryde, Australia; Gonal-f, Merck Serono, Frenchs Forest, Australia). Transvaginal oocyte aspiration (Day 0) was performed using a double lumen needle (Cook, Brisbane, Australia) under ultrasound guidance 36 h after the administration of human chorionic gonadotrophin (hCG: 10,000 IU Pregnyl, Organon, Sydney, Australia; or 500 IU Ovidrel, Merck Serono).

The male partners provided semen by masturbation within 2 h of egg collection, having been requested to have 2–4 days sexual abstinence. Sperm were isolated from semen using 50%:95% density gradients (Puresperm, Nidacon, Mölndal, Sweden), resuspended in Universal IVF (U-IVF) medium (Origio, Måløv, Denmark) and held at 37 °C until use.

2.2. Oocyte preparation and ICSI

Oocyte-cumulus-complexes (OCCs) were washed twice and then cultured in U-IVF medium at 6% CO_2 in air at 37 °C until denuding. Cumulus cells were removed by brief incubation in Synvitro Hylase (Origio) 2–4 h after oocyte collection. The oocytes free of cumulus cells were then cultured in U-IVF medium overlaid by paraffin oil (Origio) for at least 1 h prior to insemination. All oocytes at the metaphase II stage, as judged by the presence of the first polar body, had one sperm suspended in SpermSlow™ (Origio) injected using an injection needle (The Pipette Company, Adelaide, Australia).

2.3. Embryo culture and time-lapse imaging

Embryoslides® (Unisense Fertilitech) were set up and equilibrated at 6% CO_2 in air at 37 °C for at least 16 h prior to use, with each well prefilled with 25 μL ISM1 medium (Origio) and the whole slide covered by 1.2 mL paraffin oil. Immediately after ICSI, oocytes were loaded individually into microwells and

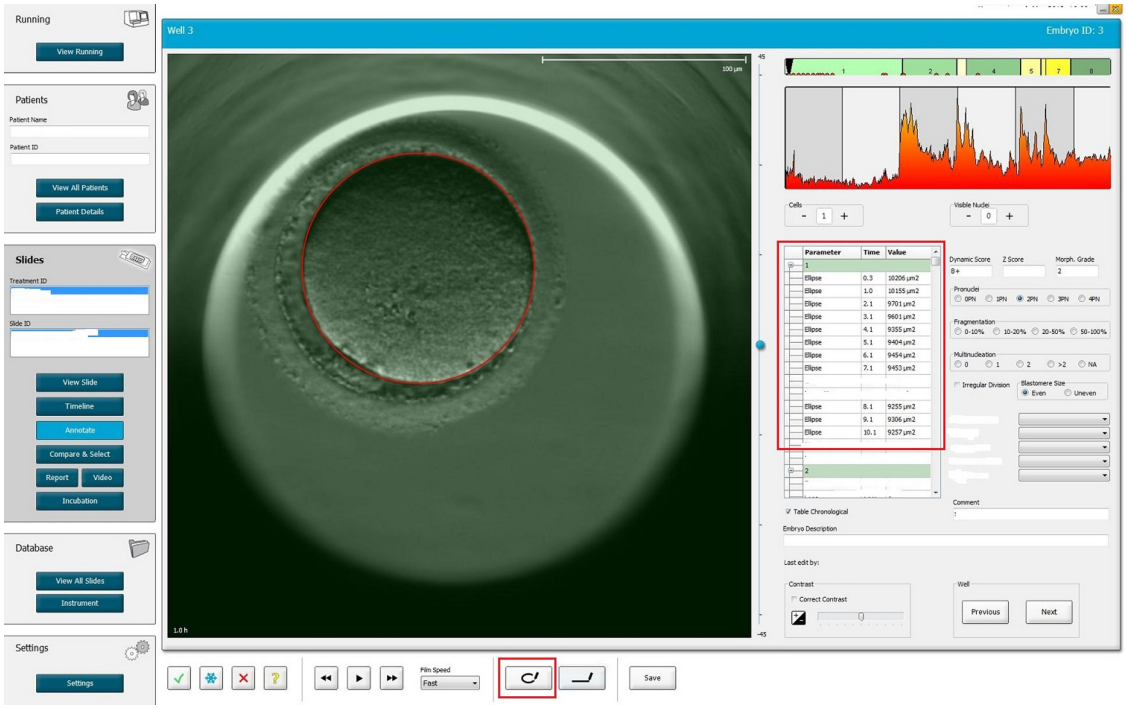

Fig. 1 – The Embryoviewer® software showing how oocyte elliptical area was measured using the ''ellipse'' function.

then inserted into the Embryoscope™ for culture (6% CO_2, 5% O_2, 89% N_2 and 37 °C) and time-lapse imaging until Day 3 when embryo transfer was performed. Transferred embryos were selected based on a morphological grading system on Day 3 as described previously [16]. Briefly, embryos were scored as 1, 2, 3, and 4, with 1 being the best and 4 the worst, and where embryos with grades 1–3 were deemed suitable for either transfer or freezing. The time-lapse imaging interval was set at 10 min with 7 focal planes scanned. Timings of biological events following ICSI (namely second polar body extrusion, pronuclear fading, and cleavage) were recorded at the nearest 10-min intervals.

2.4. *Morphometric analysis of oocytes and polar bodies*

The ''ellipse'' function of the Embryoviewer® software (Unisense Fertilitech) was employed to measure the size of each oocyte from the initial 35 treatment cycles by fitting the vitellus into a manually drawn ellipse in the program (Fig. 1). The software automatically determines a major semi-axis (a) and a minor semi-axis (b) of the ellipse which are used to calculate area (A) of the ellipse according to the formula $A = \pi ab$ (information provided by the Department of Research and Development, Unisense Fertilitech). Measurements of both the first and second polar bodies were also performed if intact and with a regular shape within these initial treatment cycles. A total of 98 polar bodies and 48 oocytes were excluded from measurement due to shape irregularities, indicating a limitation of this tool. The size of remaining oocytes was measured immediately after loading into the Embryoscope™ and then recorded hourly for up to 9 h. All measurement was performed by the same embryologist (YL) with more than 10 years of clinical embryology experience. Repeat measurement of the elliptical area of five oocytes, each 20 times, showed good reproducibility with coefficients of variation ranging between 0.41% and 0.69% (Table 1).

2.5. *Statistical analysis*

The elliptical area and timing data in this study were expressed as mean ± SEM, with groups compared using Microsoft Office Excel 2010 and the Statistical Package for the Social Sciences 20.0 (SPSS, Chicago, IL, USA) by Student's t-test (paired or unpaired as appropriate), repeated measurement ANOVA or one-way ANOVA. Relationships of linear data were analyzed via Pearson Correlation analysis. Prediction relationships were analyzed using Receiver Operating Characteristic (ROC) curves and results expressed as the area under the curve (AUC). All differences were considered significant if $p < 0.05$.

Table 1 – The reproducibility of measurements of elliptical area to represent oocyte size.

Oocyte	Elliptical area for 20 repeat measurements		
	Mean (μm^2)	SD (μm^2)	CV (%)
1	10,420.00	51.08	0.49
2	10,134.00	41.75	0.41
3	11,057.35	75.79	0.69
4	9898.40	64.15	0.65
5	10,182.75	65.07	0.64

Measurements made were the mean, standard deviation (SD) and coefficient of variation (CV).

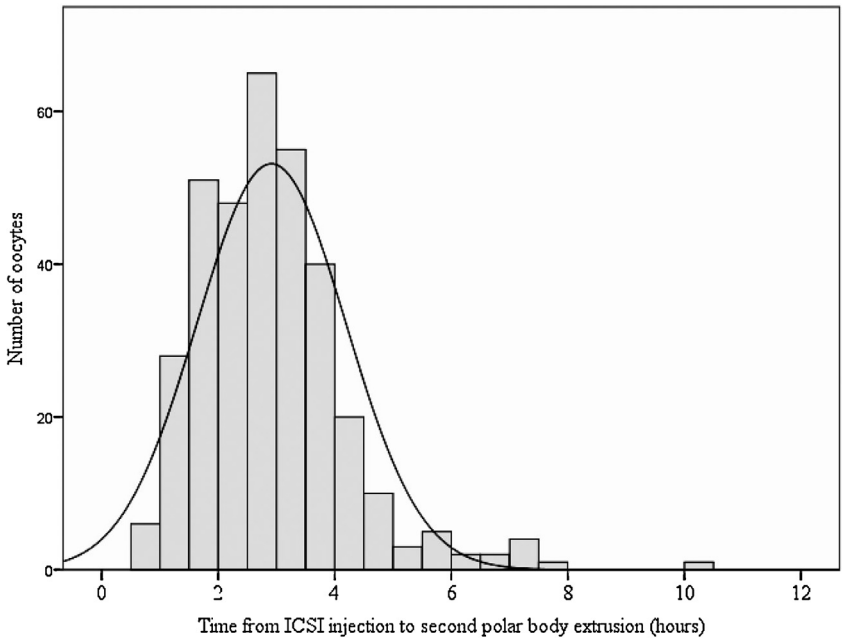

Fig. 2 – A frequency distribution of the time from the ICSI injection to the appearance of the second polar body for 341 fertilized oocytes. The bell curve shows the normal distribution over this range.

3. Results

3.1. *The second polar body*

The mean time for the appearance of the second polar body in fertilized oocytes was 2.9 ± 0.1 h after sperm injection with a range of 0.70–10.15 h as shown in Fig. 2. The elliptical area of the first and second polar bodies was measured for those with a regular shape, and the second polar body (277.1 ± 78.2 μm^2; $n = 104$) was significantly smaller ($p < 0.005$) compared to the first (358.3 ± 96.8 μm^2; $n = 108$).

3.2. *Change in size of oocytes after ICSI*

The elliptical area of the oocyte vitellus was measured in a subset of 37 unfertilized and 155 fertilized oocytes as shown in Fig. 3, with the unfertilized and fertilized oocytes coming from women of a similar age (34.6 ± 0.6 years vs. 34.4 ± 0.4 years, $p > 0.05$). Following ICSI, both fertilized and unfertilized oocytes reduced in size for the first 2 h ($p < 0.05$) despite having a similar elliptical area at starting point ($p > 0.05$), but thereafter the unfertilized stopped reduction and remained constant ($p > 0.05$) while the fertilized oocytes continued to reduce in size and became significantly smaller than their unfertilized counterparts ($p < 0.05$). However, Fig. 3 and Table 2 confirm that this divergence is around the time that the second meiotic division occurs with an associated loss of oocyte cytoplasm carried out by the second polar body. The size of the fertilized oocytes was therefore re-analyzed relative to the appearance of the second polar body during the second meiotic division as shown in Table 3. Irrespective of the time taken for second polar body extrusion, the vitellus shrinks prior to extrusion but then remains constant afterwards. This is also supported by the fact that a statistically significant correlation exists between elliptical area of the fertilized oocytes and time prior to the second polar body extrusion ($r = -0.132$, $p = 0.002$) but not after ($r = -0.005$, $p = 0.871$).

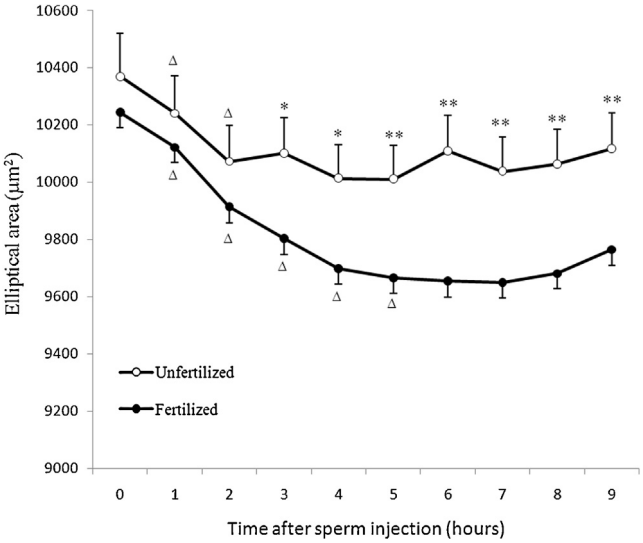

Fig. 3 – The elliptical area (mean ± SEM) of unfertilized ($n = 37$) and fertilized ($n = 155$) oocytes relative to the time of ICSI injection. No significant differences between fertilized and unfertilized oocytes at time-points "0", "1", and "2" ($p > 0.05$). *$p < 0.05$, **$p < 0.01$: fertilized and unfertilized oocytes significantly different at these time-points; $^{\Delta}p < 0.05$: elliptical area significantly different to previous time-point in that group.

Table 2 – The prediction of fertilization based upon the change in elliptical area of the oocytes from the first measurement to different time points relative to sperm injection.

Change measured at different times relative to sperm injection (h)	Receiver operator characteristics for predicting fertilization	
	AUC	*p* value
1	0.526 (0.428–0.624)	0.628
2	0.572 (0.473–0.671)	0.174
3	0.688 (0.599–0.778)	0.000
4	0.695 (0.591–0.778)	0.000
5	0.723 (0.630–0.816)	0.000
6	0.800 (0.717–0.882)	0.000
7	0.759 (0.668–0.851)	0.000
8	0.744 (0.661–0.828)	0.000
9	0.728 (0.640–0.816)	0.000

The predictive value was determined by receiver operator characteristics and expressed as the area under the curve (AUC), the 95% confidence interval and the statistical significance.

3.3. *Association between timing of second meiotic division and female age, and its effect on subsequent embryo development*

The times of second polar body extrusion, pronuclear fading and first cleavage relative to ICSI or second polar body extrusion are shown in three different age groups of patients (Table 4). Second polar body extrusion was significantly delayed in women >38 years, but similar between the medium (35–38 years) and younger groups (<35 years). No significant differences were detected in the other timing parameters measured. Analysis of morphokinetics according to the final grade of the embryo (Table 5) revealed that the second polar body was extruded at similar times in embryos with different morphological grades on Day 3, but that better quality embryos spent less time reaching pronuclear fading and first cleavage relative to either ICSI or second polar body. The prediction of embryo quality from the timing of the first cleavage division was examined using ROC and AUC as shown in Table 6, with cut-offs determined to enable identification of the good embryos. The cleavage time relative to sperm injection (cut-off 26.00 h), second polar body extrusion (cut-off 23.02 h) and pronuclear fading (cut-off 2.96 h) was significantly associated with the development of embryos suitable for transfer or freezing (grades 1, 2 and 3) whilst top grade (grade 1) embryos could be identified accurately by the time to cleavage from sperm injection (cut-off 25.40 h) and second polar body extrusion (cut-off 22.59 h) only.

4. Discussion

Embryoviewer®, the associated software of Embryoscope™, enables measurement of the size of oocyte and polar bodies by

Table 3 – The elliptical area of fertilized oocytes expressed relative to the extrusion of the second polar body.

Time after ICSI when the second polar body was extruded (hours)	*n* *	Time relative to the extrusion of the second polar body					
		Before			After		
		Initial measurement of elliptical area (μm^2; mean ± SEM)	Last measurement of elliptical area (μm^2; mean ± SEM)	*p* value**	First measurement of elliptical area (μm^2; mean ± SEM)	Last measurement of elliptical area (μm^2; mean ± SEM)	*p* value**
1.01–2.00	36	10,389.6 ± 107.2	10,207.1 ± 121.0	0.004	9863.9 ± 123.6	9937.8 ± 129.6	0.265
2.01–3.00	55	10,189.0 ± 96.8	9880.4 ± 99.3	0.000	9698.2 ± 97.8	9651.4 ± 89.7	0.232
3.01–4.00	40	10,251.9 ± 103.5	9893.0 ± 109.0	0.000	9729.6 ± 96.0	9827.3 ± 107.0	0.067
4.01–5.00	15	10,042.7 ± 142.0	9708.4 ± 145.6	0.000	9564.2 ± 134.5	9668.2 ± 145.6	0.222
5.01–8.00	7	10,345.9 ± 244.3	10,078.6 ± 281.8	0.045	9819.0 ± 277.0	9813.4 ± 299.3	0.894

Assessments were made at the first and last viewing of oocytes before and after the extrusion of the second polar body.
SEM: standard error of mean.
* Two oocytes were excluded due to extrusion of the second polar body being ≤1 h.
** Paired Students *t*-test.

Table 4 – Comparisons of timings of events up to first cleavage following ICSI between different female age groups.

Timing parameters (hours)	<35 years (*n* = 195) (mean ± SEM)	35–38 years (*n* = 76) (mean ± SEM)	>38 years (*n* = 64) (mean ± SEM)
Second polar body extrusion	2.8 ± 0.1[a]	2.8 ± 0.1[a]	3.4 ± 0.2[b]
Pronuclear fading	23.0 ± 0.3	23.1 ± 0.4	23.9 ± 0.4
First cleavage			
Relative to ICSI	26.7 ± 0.3	26.1 ± 0.5	27.0 ± 0.4
Relative to second polar body extrusion	23.9 ± 0.3	23.3 ± 0.4	23.6 ± 0.4

Only embryos having reached 2-cell stage or beyond by Day 3 were included for analysis. Second polar body extrusion and pronuclear fading were relative to sperm injection, and the first cleavage division relative to both sperm injection and extrusion of the second polar body.
SEM: standard error of mean.
One-way ANOVA followed by LSD post hoc test.
Different superscripts in the same row indicate statistical significance ($p < 0.01$).

Table 5 – Comparisons of timings of events up to first cleavage following ICSI between different morphological grades of embryos on Day 3.

Timing parameters (hours)	Embryo grade			
	Grade 1 (n = 97) (mean ± SEM)	Grade 2 (n = 97) (mean ± SEM)	Grade 3 (n = 37) (mean ± SEM)	Grade 4 (n = 104) (mean ± SEM)
Second polar body extrusion	2.9 ± 0.1	2.9 ± 0.1	2.8 ± 0.2	3.0 ± 0.1
Pronuclear fading	22.3 ± 0.2^{a}	22.8 ± 0.3^{ab}	23.8 ± 0.8^{bc}	24.0 ± 0.4^{c}
First cleavage				
Relative to ICSI	25.3 ± 0.3^{a}	25.9 ± 0.4^{ab}	27.1 ± 0.9^{bc}	28.3 ± 0.5^{c}
Relative to second polar body extrusion	22.4 ± 0.2^{a}	23.0 ± 0.3^{ab}	24.3 ± 0.8^{bc}	25.3 ± 0.5^{c}

Grade 1 denotes the best and grade 4 the worst embryo quality. Grades 1, 2 and 3 were suitable for transfer or cryopreservation. Only embryos having reached 2-cell stage or beyond were included for analysis.
SEM = Standard error of mean.
One-way ANOVA followed by LSD post hoc test.
Different superscripts in the same row indicate statistical significance ($p < 0.05$).

Table 6 – The prediction of embryo quality from the time to the first cleavage division.

Time to first cleavage from	Receiver operator characteristics for prediction of embryo quality			
	Top grade		Transfer/freeze	
	AUC	p value	AUC	p value
Sperm injection	0.596 (0.533–0.660)	0.006	0.638 (0.573–0.703)	0.000
Second polar body extrusion	0.601 (0.537–0.665)	0.004	0.632 (0.566–0.699)	0.000
Pronuclear fading	0.552 (0.487–0.618)	0.132	0.633 (0.566–0.701)	0.000

The predictive value was determined by receiver operator characteristics and expressed as the area under the curve (AUC), the 95% confidence interval and the statistical significance.
Time to cleavage was examined relative to three starting points, namely sperm injection, second polar body extrusion and pronuclear fading.
Embryos were classified as top grade (grade 1) or suitable for either transfer or freezing (grades 1, 2 or 3).

using an elliptical tool (Fig. 1) but it is only suitable for evenly shaped cells. The present study was unable to measure the size of 98 polar bodies and 48 oocytes because of shape irregularities. The fitting of the ellipse to the cell requires practice, but a high degree of precision can be achieved with small coefficients of variation being obtained (0.41–0.69%; Table 1). In the present study, all measurement was performed by the same embryologist, which minimized potential operator-related variability. However, the future development of computer software to measure the area of the oocyte vitellus, being independent of shape regularity, would improve the efficacy and accuracy of this measurement.

The elliptical tool of the Embryoviewer® was applied to a subset of oocytes with two polar bodies and the second polar body was found to be consistently smaller than the first. The elliptical area of oocytes was also measured in both fertilized and unfertilized oocytes. The original time-lapse study from the Adelaide group [5] reported a reduction in oocyte diameter following ICSI from injection (111.6 ± 3.6 μm) to pronuclei abuttal (108.4 ± 3.4 μm) and then 17 h post-injection (106.0 ± 4.6 μm) but the difference was only statistically significant between the time at injection and 17 h later. Whilst pioneering work at the time, there were two limitations to this study. Firstly, the sample size was relatively small as only 38 fertilized oocytes were included for observations, largely due to the physical constraints of the video system being able to film only one egg at a time. Secondly, only the three time points mentioned above were reported so that detailed changes in oocyte size between these time points were not available. Nevertheless, despite the above limitations, it was the findings of Payne et al. [5] that inspired us to re-explore with the benefit of current day technology the subtle changes in the size of human oocytes following ICSI. The fertilized oocytes in the present study had a significantly reduced elliptical area compared to the unfertilized oocytes, starting from 3 h until 9 h following ICSI as shown in Fig. 3 and Table 2. Considering the similar response in size of the fertilized and the unfertilized oocytes immediately after ICSI, it would appear that the physical presence of the sperm injected may well trigger some early cytoplasmic events such as calcium oscillations in both groups of oocytes [17–19]. Subsequent biological events triggered by the calcium oscillation such as the cortical reaction could also be linked with the size change, where the contents of cortical granules are released into the perivitelline space to form a cortical granule envelope, which is present until hatching at the blastocyst stage [20]. The point at which the second meiotic division is complete is clearly associated with the cessation of size change in fertilized oocytes. This is not due to the loss of cytoplasm in the second polar body since analysis was separated to time before and after second polar body extrusion, and was irrespective of the time at which the second polar body extrusion occurred relative to injection (Table 3).

Regulation of oocyte size has been confirmed in animal studies where in vitro cultured oocytes and embryos were able to adjust their volume to deal with a change in environment osmolarity by employing a range of osmolyte regulation systems [21,22]. The switch between different osmolyte

transporting systems was postulated to occur in between major biological events such as ovulation, fertilization or the end of 2-cell stage [21]. Therefore a change to another volume regulating system might account for the cessation of oocyte size reduction at the resumption of meiosis. Despite the strong correlation between oocyte shrinkage and fertilization, there did not appear to be any relationship between embryo development and second polar body extrusion timing as shown in Table 5 (expressed by morphological grade on Day 3 in the present study), although advanced female age (>38 years) was seen with delayed second polar body extrusion.

The occurrence of the second meiotic division, as indicated by the extrusion of the second polar body, is an important milestone during the fertilization process and was previously reported to take place over a wide time range of 1–8.00 h post ICSI [1,2,4,5,12]. Results in the present study indicate a similarly wide range of timings (0.70–10.15 h) following ICSI of nuclear mature oocytes, and this could possibly be explained by the aspiration of human ovarian follicles at a set time relative to the ovulatory trigger resulting in the collection of oocytes that are at different stages of nuclear and cytoplasmic maturity [23,24], with the double lumen aspiration system with follicle flushing helping retrieve oocytes from smaller follicles. This variable time to the resumption of the second meiotic division supports the proposal that embryo morphokinetic timings be made relative to a biological point of reference rather than a procedural reference point [10], and this biological reference point could in fact be any feature from second polar body extrusion onwards. Whilst the choice of pronuclear fading has the advantage that it can also be applied to oocytes fertilized by IVF overnight and placed in the Embryoscope™ after checking for the presence of pronuclei the following day [10,25], the value of expressing times relative to the second polar body should not be ignored (Table 6). This is consistent with the observations that the early occurrence of the first zygotic cleavage has been shown to be a useful predictive marker of implantation potential in human embryos [14,15] and mammalian embryos in general [26]. A shorter time to the completion of the second meiotic division was initially suggested by Payne et al. [5] to be a marker for the development of good quality embryos, possibly due to this reflecting improved oocyte maturity at collection, although this is not always seen [8,12] and nor was it seen in the present study. Whilst the expression of embryo morphokinetics relative to sperm injection in ICSI cycles is the current norm [6], future prospective studies with larger numbers comparing different starting points applied to the full range of embryo morphokinetic parameters would seem important to maximize the predictive value of time-lapse videography. The present study provided preliminary data based on embryo quality as the end-point due to a limited number of clinical cases and the occurrence of several two embryo transfers, and so future studies using large numbers of known implantation data are required. Another limitation of the present study includes the unavailability of more detailed demographic information of female patients such as antral follicle count and anti-Müllerian hormonal level.

In conclusion, (i) the second polar body of human oocytes extrudes at various times following sperm injection and is smaller than the first polar body, (ii) the timing of second polar body extrusion is significantly delayed ($p < 0.01$) when female age is more than 38 years old, but not related to subsequent embryo development, (iii) all human oocytes reduce in size following sperm injection, (iv) completion of the second meiotic division in the fertilized oocytes is a pivotal event in terminating shrinkage of the vitellus, and (v) a shorter time to the first cleavage division either from sperm injection or extrusion of the second polar body is a significant predictive marker for embryo quality on Day 3.

Conflict of interest

None declared.

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REFERENCES

[1] Suppinyopong S, Choavaratana R, Karavakul C. Timing of second polar body extrusion and pronuclear formation after intracytoplasmic sperm injection (ICSI). J Med Assoc Thai 2000;83(5):500–3.
[2] Nagy ZP, Liu J, Joris H, Devroey P, Van Steirteghem A. Time-course of oocyte activation, pronucleus formation and cleavage in human oocytes fertilized by intracytoplasmic sperm injection. Hum Reprod 1994;9(9):1743–8.
[3] Capmany G, Taylor A, Braude PR, Bolton VN. The timing of pronuclear formation. DNA synthesis and cleavage in the human 1-cell embryo. Mol Hum Reprod 1996;2 (5):299–306.
[4] Mio Y, Maeda K. Time-lapse cinematography of dynamic changes occurring during in vitro development of human embryos. Am J Obstet Gynecol 2008;199(6):660. e1–e5.
[5] Payne D, Flaherty SP, Barry MF, Matthews CD. Preliminary observations on polar body extrusion and pronuclear formation in human oocytes using time-lapse video cinematography. Hum Reprod 1997;12(3):532–41.
[6] Meseguer M, Herrero J, Tejera A, Hilligsoe KM, Ramsing NB, Remohi J. The use of morphokinetics as a predictor of embryo implantation. Hum Reprod 2011;26(10):2658–71.
[7] Meseguer M, Rubio I, Cruz M, Basile N, Marcos J, Requena A. Embryo incubation and selection in a time-lapse monitoring system improves pregnancy outcome compared with a standard incubator: a retrospective cohort study. Fertil Steril 2012;98(6):1481–9. e10.
[8] Azzarello A, Hoest T, Mikkelsen AL. The impact of pronuclei morphology and dynamicity on live birth outcome after time-lapse culture. Hum Reprod 2012;27(9):2649–57.
[9] Aguilar J, Motato Y, Escriba MJ, Ojeda M, Munoz E, Meseguer M. The human first cell cycle: impact on implantation. Reprod Biomed Online 2014;28(4):475–84.
[10] Cruz M, Garrido N, Gadea B, Munoz M, Perez-Cano I, Meseguer M. Oocyte insemination techniques are related to alterations of embryo developmental timing in an oocyte donation model. Reprod Biomed Online 2013;27(4):367–75.

[11] Kirkegaard K, Kesmodel US, Hindkjaer JJ, Ingerslev HJ. Time-lapse parameters as predictors of blastocyst development and pregnancy outcome in embryos from good prognosis patients: a prospective cohort study. Hum Reprod 2013;28(10):2643–51.
[12] Van den Bergh M, Bertrand E, Englert Y. Second polar body extrusion is highly predictive for oocyte fertilization as soon as 3 hr after intracytoplasmic sperm injection (ICSI). J Assist Reprod Genet 1995;12(4):258–62.
[13] Nicoli A, Palomba S, Capodanno F, Fini M, Falbo A, La Sala GB. Pronuclear morphology evaluation for fresh in vitro fertilization (IVF) and intracytoplasmic sperm injection (ICSI) cycles: a systematic review. J Ovarian Res 2013;6(1):64.
[14] Van Montfoort AP, Dumoulin JC, Kester AD, Evers JL. Early cleavage is a valuable addition to existing embryo selection parameters: a study using single embryo transfers. Hum Reprod 2004;19(9):2103–8.
[15] Salumets A, Hyden-Granskog C, Makinen S, Suikkari AM, Tiitinen A, Tuuri T. Early cleavage predicts the viability of human embryos in elective single embryo transfer procedures. Hum Reprod 2003;18(4):821–5.
[16] Alpha Scientists in Reproductive Medicine, Embryology ESIGo. The Istanbul consensus workshop on embryo assessment: proceedings of an expert meeting. Hum Reprod 2011;26(6):1270–83.
[17] Yoon SY, Jellerette T, Salicioni AM, Lee HC, Yoo MS, Coward K, et al. Human sperm devoid of PLC, zeta 1 fail to induce Ca (2+) release and are unable to initiate the first step of embryo development. J Clin Invest 2008;118(11):3671–81.
[18] Kashir J, Heindryckx B, Jones C, De Sutter P, Parrington J, Coward K. Oocyte activation, phospholipase C zeta and human infertility. Hum Reprod Update 2010;16(6):690–703.
[19] Dozortsev D, Rybouchkin A, De Sutter P, Qian C, Dhont M. Human oocyte activation following intracytoplasmic injection: the role of the sperm cell. Hum Reprod 1995;10(2):403–7.
[20] Dandekar P, Talbot P. Perivitelline space of mammalian oocytes: extracellular matrix of unfertilized oocytes and formation of a cortical granule envelope following fertilization. Mol Reprod Dev 1992;31(2):135–43.
[21] Baltz JM, Tartia AP. Cell volume regulation in oocytes and early embryos: connecting physiology to successful culture media. Hum Reprod Update 2010;16(2):166–76.
[22] Tartia AP, Rudraraju N, Richards T, Hammer MA, Talbot P, Baltz JM. Cell volume regulation is initiated in mouse oocytes after ovulation. Development 2009;136(13):2247–54.
[23] Balakier H, Sojecki A, Motamedi G, Librach C. Time-dependent capability of human oocytes for activation and pronuclear formation during metaphase II arrest. Hum Reprod 2004;19(4):982–7.
[24] Sundstrom P, Nilsson BO. Meiotic and cytoplasmic maturation of oocytes collected in stimulated cycles is asynchronous. Hum Reprod 1988;3(5):613–9.
[25] Dal Canto M, Coticchio G, Mignini Renzini M, De Ponti E, Novara PV, Brambillasca F, et al. Cleavage kinetics analysis of human embryos predicts development to blastocyst and implantation. Reprod Biomed Online 2012;25(5):474–80.
[26] Lechniak D, Pers-Kamczyc E, Pawlak P. Timing of the first zygotic cleavage as a marker of developmental potential of mammalian embryos. Reprod Biol 2008;8(1):23–42.