Relationship between meiotic spindle characteristics in human oocytes and the timing of the first zygotic cleavage after intracytoplasmic sperm injection

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Abstract

Purpose To investigate the relationship between meiotic spindle characteristics in human oocytes and the timing of the first zygotic cleavage after intracytoplasmic sperm injection (ICSI).

Methods Zygotes that had cleaved to two-cell stage by 27 h post-ICSI were classified as early cleaving and the remainder as late cleaving. Meiotic spindle parameters previously imaged using the PolScope were compared between the two groups.

Results Of 384 embryos, 163 were classed as early cleaving and 221 as late cleaving. The rate of blastocyst formation or pregnancy by Day 2 embryo transfer was significantly higher following early cleavage than after late cleavage (52.4% vs. 24.4% or 32.6% vs. 11.4%). Spindle areas (108.0 vs. 89.8 μm^2), lengths (14.7 vs. 13.4 μm) and PolScope retardance were also significantly greater in the early cleaving group.

Conclusions Meiotic spindle parameters determine the timing of the first zygotic cleavage and are strong indicators of human embryo developmental potential.

Keywords Early cleavage · Meiotic spindle · Embryo quality · PolScope · Human oocytes

Introduction

In the treatment of human infertility, overall pregnancy rates have improved over the years, but the birth rate in assisted reproduction programs is still relatively low, despite the transfer of more than one embryo in many centers. The transfer of several embryos has led to a high rate of multiple pregnancies following IVF and ICSI [1]. To prevent this adverse outcome, many recent studies have focused on elective single embryo transfer (eSET) [2, 3]. Therefore, one of the greatest problems in assisted reproduction technology today relates to the selection of the optimal embryos for transfer, to achieve high pregnancy rates without increasing multiple pregnancy rates.

In some IVF centers, embryos are cultured until the blastocyst stage to facilitate selection. However, several studies have been unable to demonstrate that blastocyst transfer is a better alternative for transfer of cleavage stage embryos [4] and the former is associated with increased risk of monozygotic twin pregnancy [5, 6]. At present, the principal method of selecting the most viable cleavage-stage embryo is based on its morphology and developmental stage on the day of transfer [7–10]. That is, embryos for transfer are chosen on the basis of their zygote and cleavage stage morphologies, including the number and equality of the blastomeres and the presence and grade of fragmentation. However, these indicators depend on subjective judgments. Another easily determined indicator of embryo quality is the timing of the first zygotic cleavage. Early cleaving zygotes are more likely to develop to the

Capsule A relationship exists between post-ICSI meiotic spindle characteristics, the timing of zygotic cleavage, and blastocyst formation. This may assist selection of competent embryos for transfer.

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blastocyst stage than their late-cleaving counterparts. This non-invasive marker of embryo developmental potential has been successfully applied in human IVF programs for identifying embryos of superior quality [11, 12]. However, the mechanisms responsible for variation in the timing of the first zygotic cleavage have not been identified.

Recently, the meiotic spindle in human oocytes has been visualized in a non-invasive manner using polarized light microscopy. Using this technique, the position of the meiotic spindle relative to the polar body was examined in relation to oocyte developmental potential after ICSI [13], and the use of spindle imaging as a marker for optional timing of ICSI [14] was investigated. These studies indicated that visualization of the meiotic spindle may be useful for the evaluation of oocyte quality. However, the relationships between the parameters of the spindle image and the timing of zygotic cleavage remain unclear.

This study aimed to investigate the association between meiotic spindle characteristics in human oocytes and the timing of the first zygotic cleavage after ICSI.

Materials and methods

Patients

This study analyzed 509 oocytes possessing the first polar body, obtained from 150 patients. The mean patient age was 38.3 ± 4.1 years ($\pm$ SD; range 27–46). The infertility etiologies of the cycles were as follows: male factor, tubal, uterine, diminished ovarian reserve, unexplained, or combined. The distribution of these etiologies was similar between groups. The study protocol and the need to perform ICSI and PolScope imaging analysis were explained to the couples and they were recruited into the study after their approval. The present study was approved by the Institutional Review Board (IRB), IVF Nagata Clinic.

Stimulation and oocyte retrieval

Ovarian stimulation was achieved using human menopausal gonadotropins (HMG; Humegon; Japan Organon, Osaka, Japan) in various protocols, but primarily the short protocol. In a small number of cycles the ultra-short protocol (minimal stimulation protocol) was used. The short and ultra-short protocols involved down-regulation with gonadotropin-releasing hormone analogues. Minimal stimulation protocols were used that employed a combination of clomiphene citrate and gonadotropins. Human chorionic gonadotropin (HCG; Mochida Pharmaceutical, Tokyo, Japan) at a dose of

10,000 IU was administered after one or two follicles of ≥ 18 mm were visualized in the ultrasound scan. Oocyte retrieval was scheduled 36 h after HCG administration. Oocytes were recovered transvaginally with ultrasound guidance. After brief exposure to 80 IU/ml hyaluronidase (Sage BioPharma, Bedminster, NJ, USA), cumulus cells were removed by repeated gentle aspiration. The denuded oocytes were then assessed with respect to their meiotic maturation status. In preparation for ICSI, oocytes with an extruded first polar body (PB1) (presumably at the metaphase II stage) were selected for meiotic spindle detection and PB1 morphology assessment. Oocytes without a polar body or presenting a germinal vesicle (GV stage) were excluded from the study.

Visualization of spindle using the PolScope and Intracytoplasmic Sperm Injection (ICSI)

For spindle imaging, each oocyte was placed in a 10 μ l drop of HEPES-buffered medium (Irvine Scientific, Santa Ana, CA, USA) covered with mineral oil (FertiPro N.V., Beernem, Belgium) on a glass bottomed culture dish (FluoroDish; World Precision Instruments Inc, Sarasota, FL, USA), which was maintained at 37°C on a heated stage. Oocytes were imaged using an Olympus IX70 inverted microscope equipped with the LC PolScope (the polarized light microscope, CRI, Woburn, MA, USA). The microscope was set up and the background calibrated according to the manufacturer's protocol. Spindle images were acquired at x400 magnification and the location, area and length of the meiotic spindle, and mean retardance of light by birefringent spindles, in oocytes were saved in the image processing system for later analysis. To optimize visualization of the spindle and polar body, oocytes were rotated using the holding pipette. After imaging, ICSI was performed with the spindle at the 6 or 12 o'clock position irrespective of polar body position. In the oocytes without detectable spindles, ICSI was performed after the first polar body was placed at the 6 or 12 o'clock position. After ICSI, the oocytes were washed and cultured separately in droplets of Global medium (LifeGlobal, Guelph, ON, Canada) supplemented with 20% Serum Protein Substitute (SPS; SAGE/Cooper Surgical, CT, USA) for examination of fertilization.

Assessment of fertilization

Oocytes were examined under an inverted microscope for fertilization 16–18 h after ICSI. Oocytes with two pronuclei and a second polar body were considered to be normally

fertilized. The zygotes were transferred daily to fresh droplets of Global medium for 3 days post-ICSI.

Assessment of the first zygotic cleavage and blastocyst culture

The first mitotic division was checked 27 h after ICSI. Zygotes that had reached the two-cell stage at this point were designated as ‘early cleaving embryos’. Zygotes that had not yet reached the two-cell stage and cleaved later than 27 h post-ICSI were classified as ‘late cleaving embryos’ [12]. After the first check at 27 h post ICSI, zygotes were observed to determine the late cleaving embryos at 43 h post ICSI. Each zygote was cultured in 20 µl droplets of Global medium separately from day 1 to day 5 at 37°C in an atmosphere of 5% O₂, 5%CO₂ and 90% N₂, and was evaluated to development to the blastocyst stage.

Transfer of day 2 embryos in the early and late cleaving groups

After the first and second checks at 27 and 43 h post ICSI, the early and late cleaving embryos were determined. Both groups were cultured for 2 days after ICSI. Day 2 embryos (4-cell stage) in the early and late cleaving groups were transferred to the patients. In this study, pregnancy rates in the early and late cleaving groups were summarized when only one embryo was transferred. Pregnancy was determined by serum HCG test on day 14 after embryo transfer. The clinical pregnancy was defined as the presence of intrauterine gestational sac at ultrasound examination 5–6 weeks after oocyte retrieval.

Statistical analysis

Statistical analysis was performed using GraphPad Prism 5.0 software (GraphPad Inc., San Diego, CA, USA). For comparison between groups, Student’s *t*-test was used for continuous variables and the chi-square test was used for binary variables. The differences were considered to be significant when $P < 0.05$.

Results

Characteristics of oocytes with or without spindle image by the PolScope

Table 1 summarizes the data for all of the oocytes visualized using the PolScope. A meiotic spindle was observed in 468 oocytes (92.0%), but not in the remaining 41 (8.0%). After ICSI, the fertilization rate in the oocytes with a visible spindle was 82.1% (384/468), and the

Table 1 Summary data for all oocytes observed using the PolScope after intracytoplasmic sperm injection

Parameter	Spindle image		
	Detected	Not detected	<i>P</i> value
No. of MII oocytes (%)	468 (92.0)	41 (8.0)	–
No. of fertilized oocytes (%)	384 (82.1)	26 (63.4)	<0.01
No. of early cleaving embryos (%)	163 (42.4)	5 (19.2)	<0.05
No. of late cleaving embryos (%)	221 (57.6)	21 (80.8)	<0.05

Fertilized zygotes that had cleaved to the two-cell stage by 27 h post-injection were designated as ‘early cleaving’, while those that not yet reached the two-cell stage were designated as ‘late cleaving’

P values indicate significant differences between the percentages in the ‘spindle detected’ and ‘spindle not detected’ embryos (Chi square test)

percentage was significantly higher than that in the oocytes without a spindle (63.4%, 26/41). Of the 384 zygotes in the spindle-detected group, 163 (42.4%) had cleaved by 27 h post-ICSI (early cleaving), and 221 (57.6%) were cleaved after 27 h of ICSI (late cleaving). In the no-spindle group, the percentage of early cleaving (19.2%, 5/26) was significantly lower than that in the spindle-detected group.

Meiotic spindle characteristics between early and late cleaving groups

Table 2 presents the spindle characteristics of the oocytes classified as early or late cleaving and Fig. 1 shows representative images of the spindles in these two groups. The spindle image in the early cleaving group (Fig. 1A and a) was more distinct and larger than that in the late cleaving group (Fig. 1B and b). Oocyte diameter and the angle between the spindle and the first polar body were similar in the two groups. Spindle lengths and areas of the early

Table 2 Comparison of oocyte spindle images in early and late cleaving embryos

Parameter	Early cleaving	Late cleaving	<i>P</i> value
No. of M II oocytes	163	221	–
Oocyte diameter (µm)	107.7±3.4	107.3±3.5	NS
Spindle to polar body angle (degrees)	29.0±27.2	24.3±24.8	NS
Spindle length (µm)	14.7±2.0	13.4±2.2	<0.01
Spindle area (µm ²)	108.0±17.8	89.8±19.2	<0.01
Spindle retardance (nm)	2.9±0.5	2.5±0.7	<0.01

Fertilized embryos that had cleaved to the two-cell stage by 27 h post-injection were designated as ‘early cleaving’, while those that not yet reached the two-cell stage were classified as ‘late cleaving’

Values are means ± SD; *P* values indicate significant differences between early and late cleaving embryos (Students *t*-test)

Fig. 1 Meiotic spindle of human oocytes imaged by the PolScope in the early and late cleavage groups. **A** An oocyte from the early cleaving group with an obvious meiotic spindle located at the 12 o'clock position. The spindle image is shown at higher magnification in inset (**a**). **B** An oocyte from the late cleaving group with an indistinct meiotic spindle located at the 12 o'clock position. The spindle image is shown at higher magnification in inset (**b**). Pb is a first polar body. Scale bars represent 50 μm

cleaving group were significantly greater ($P<0.01$) than that in the late cleaving group (14.7 ± 2.0 vs. 13.4 ± 2.2 μm , and 108.0 ± 17.8 vs. 89.8 ± 19.2 μm^2). Furthermore, spindle retardance in early cleaving group was significantly higher than that in late cleaving group ($P<0.01$).

Blastocyst development in the early and late cleaving groups

For evaluation of developmental competence to the blastocyst stage, 82 of the early cleaved embryos and 45 of the late cleaved embryos were cultured in Global medium. As shown in Fig. 2, the percentage of embryos that developed to blastocyst stage in the early cleaving group (52.4%) was significantly higher ($P<0.01$) than that in the late cleaving group (24.4%).

Fig. 2 Comparison of blastocyst rates between the early and late cleaved human embryos. The percentage of embryos that developed to blastocyst stage in the early cleaving group was significantly higher than in the late cleaving group (Chi-squared test, $P<0.01$). The actual percentages are shown within the bars

Pregnancies in the early and late cleaving groups

Figure 3 shows the pregnancy rates of Day 2 embryos in the early and late cleaving groups. The percentage of clinical Pregnancies in the early cleaving group (32.6%; 15/46) was significantly higher than ($P<0.05$) than that in the late cleaving group (11.4%; 4/35).

Discussion

Previous studies have observed that human embryos that cleaved earlier to the 2-cell stage exhibited a higher rate of blastocyst formation [15, 16] compared with late cleaving embryos, and that transfer of the early cleaving embryos led to significantly higher implantation and pregnancy rates

Fig. 3 Comparison of pregnancy rates between the early and late cleaved human embryos. Pregnancy rate by transfer of Day 2 embryos in the early cleaving group was significantly higher than that in the late cleaving group (Chi-squared test, $P<0.05$). The actual percentages are shown within the bars

[11, 12, 17, 18]. This study confirmed that embryos that had cleaved by 27 h had significantly higher blastocyst rates than later cleaving embryos. However, the mechanism regulating the timing of the first zygotic cleavage remains incompletely understood. The early cleaving embryos may simply have been fertilized earlier but the use of ICSI restricts the time for sperm incorporation to a relatively short period. It has been shown that in humans the timing of the first zygotic cleavage is not influenced by the timing of fertilization or by semen parameters [19]. The molecular and genetic condition of gametes and, consequently, the zygote are expected to affect the duration of the first cell cycle. Maternal genes and proteins inherited from the oocyte presumably regulate the first cleavage. The state of the ooplasmic organelles, such as mitochondria efficiency and ATP content, may also affect the timing of the first zygotic cleavage [20].

In the present study, we observed that, in human oocytes, the timing of the first zygotic cleavage after ICSI was significantly related to meiotic spindle parameters. The spindle apparatus is an essential cellular organelle, crucial for the high fidelity of chromosome segregation during the meiotic divisions of oogenesis [21]. The meiotic spindle has been extensively investigated as a possible predictive feature for oocyte selection but most of these earlier studies have been performed using fluorescent microscopy [22]. Although this technique provides detailed information about chromosomes and meiotic spindles, its clinical use is limited by its invasive nature and its inability to be used for studying live oocytes. Imaging of the meiotic spindle in live oocytes has been made possible by the recent development of an orientation-independent polarized light microscope, the PolScope, which allows the non-invasive study of spindle architecture in live human oocytes, without affecting their viability [23].

So far, it has been reported that the presence of a birefringent meiotic spindle in human oocytes observed using the PolScope is associated with higher fertilization and pregnancy rates [14, 24–26]. The location of the meiotic spindle close to the first polar body was correlated with fertilization and cleavage rates [25] and embryo quality [27]. Additionally, the light retardance by human oocyte spindles was positively correlated with the pronuclear score after ICSI [28]. Pregnancy rate was also strongly correlated with the retardance of meiotic spindle [29]. The magnitude of the retardance of the polarized light provides an indication of the density, high order alignment, or thickness of a birefringent object [28, 29]. Our results showed that the areas and lengths of the spindles of early cleaving oocytes were greater than those in late cleaving oocytes. Moreover, the retardance of the meiotic spindle in the early cleaving group was significantly higher than that in the late group. Thus, we conclude that meiotic spindle

characteristics strongly influence the timing of the first zygotic cleavage after ICSI.

It is apparent from this study that some meiotic spindle parameters might be effective for the selection of good quality oocytes. Therefore, in the near future, we must evaluate the efficiency of using spindle parameter criteria for the selection of good quality embryos characterized by early cleavage.

In conclusion, meiotic spindle characteristics are significantly associated with early or late cleavage. These results suggest that quantitative measurement of meiotic spindle parameters may be valuable for identifying human embryo developmental potential in the IVF laboratory.

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