Visualization of meiotic spindle and subsequent embryonic development in in vitro and in vivo matured human oocytes

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Abstract

Purpose The aim of this study is to investigate the relationship between spindle location and embryonic development of in vivo and in vitro matured human oocytes.

Methods The spindles of 134 *in vivo* matured, 105 *in vitro* matured oocytes were examined by Polscope at the time of ICSI.

Results The spindles were visualized in 83.6 and 77.1% of in vivo and in vitro matured oocytes respectively. The rate of fertilization of in vivo matured oocytes with spindles beneath or adjacent to the first polar body (angle of 0–5°) was significantly higher (93.3%) than all other groups. The proportions of various spindle positions did not differ statistically in in vivo and in vitro matured oocytes.

Conclusions Meiotic spindle location with regard to the first polar body appears to influence fertilization rate.

Keywords ICSI · IVF · Polar body · Spindle

Introduction

Meiotic spindle of human oocytes cannot be visualized in living cells under conventional microscopes. The development of the Polscope enables us to observe the spindle non-invasively [1]. During ICSI, the location of the first polar body is commonly used as an indication of the spindle position, with the assumption that they are located in close proximity. To avoid damage to the spindle, oocytes are injected at the 3 o'clock position with the first polar body at the 6 or 12 o'clock position. However, with the aid of the Polscope, some reports suggest that the location of the first polar body does not necessarily correspond to the spindle position [2, 3]. Moreover, oocytes with invisible meiotic spindles have lower fertilization rate and poorer developmental potential. Thus, spindle positions may be used to predict the quality of oocytes. The reasons for the displacement of the spindle are not understood. Rienzi et al. [4] suggested that PB displacement during manipulations for cumulus removal results in various spindle relocations angled from the first PB in human oocytes. In contrast, another research pointed out that mechanical manipulation cannot account for the various spindle locations observed in MII oocytes. They observed different spindle positional distributions in in vivo and in vitro matured mouse oocytes, which may reflect differences in their cytoplasmic maturities, and they postulated that the spindle displacement is a time-dependent process that involves a deficiency in GV oocyte maturation by unknown factors [5]. Battagli et al. [6] found that the proportion of oocytes with abnormal meiotic spindles was significantly higher in older women than in younger women and concluded that abnormal spindles may contribute to the high prevalence of aneuploidy in embryos from this population.

In this study, Spindle locations did not show significant difference between in vivo and in vitro matured oocytes, but seemed to influence the rate of fertilization.

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In this study, we compared spindle locations in *in vivo* and *in vitro* matured MII human oocytes and investigated whether the spindle position could be related to the fertilization and the development of oocytes.

Materials and methods

In vivo matured oocytes

From April 2005 to January 2007, meiotic spindles of oocytes from 15 patients with male factor infertility requiring ICSI were included. Each woman underwent controlled ovarian stimulation with a long protocol of GnRH agonist. FSH (Gonal-F; Serono, Switzerland) was used for ovarian stimulation. Follicular growth was monitored with vaginal ultrasonography and serum estradiol (E_2) levels. When the leading follicles reached a mean diameter of 18 mm with proportional serum E_2 levels, 10 000 IU of HCG (Profasi, Serono) was given. Oocytes were retrieved 36 h later under the guidance of vaginal ultrasound. The oocyte–cumulus complexes were cultured in human tubal fluid (HTF) medium with 10% serum protein substitute (SPS, SAGE, USA) and incubated at 37°C in an atmosphere of 6% CO_2 (to maintain the medium at pH 7.20–7.25) in air for 2–4 h before ICSI. A control group of 15 cases with similar indication was chosen randomly. They were treated under the same protocol, but oocytes were injected without Polscope during ICSI.

In vitro matured oocytes

Eleven patients with polycystic ovary syndrome (PCOS) were treated with natural cycle of IVM–ICSI. The patients were given 10,000 IU hCG when the dominant follicle reached 12 mm in diameter and the thickness of endometrium was >6 mm. Oocytes were collected 36 h after hCG administration. Immature oocytes were cultured in LAG medium (Medicult IVM, Denmark) for 2 to 3 h, then transferred to IVM medium (Medicult IVM, Denmark) containing 10% maternal serum, 0.075 IU/ml FSH (Gonal-F, Serono, Switzerland) and 0.1 IU/ml hCG (Profasi, Serono, Switzerland) for another 28–32 h. Oocytes were then denuded, spindles visualized and ICSI performed, as in *in vivo* matured oocytes.

ICSI and visualization of meiotic spindles

Oocytes were exposed to hyaluronidase and aspirated in and out of fine hand-pulled Pasteur pipettes repeatedly to remove surrounding cumulus and corona cells. ICSI was performed on mature oocytes, which had the first polar body extruded. At the time of ICSI, Polscope and asso-

ciating software (SpindleView, USA) was used to examine the spindles, and the oocytes were divided into five groups according to the angle between the spindles and the first polar body. Groups I, II, III and IV referred to oocytes with angle of 0–5°, 6–45°, 46–90°, and >90° respectively. Oocytes with no spindle detected were classified as group V. ICSI was performed avoiding the spindle at the site of entry, usually at 3 o'clock position (the first polar body at 6 or 12 o'clock position).

Embryo culture and transfer

Fertilization and survival situation was evaluated 16 to 18 h after ICSI. Zygotes with two pronuclei were judged as fertilized. Embryos were graded 72 h post-insemination, and those with more than six blastomeres of equal sizes and less than 20% fragmentation were defined as top quality embryos. These were chosen for embryo transfer, with a maximum of three embryos replaced, two for patients under the age of 35 undergoing the first treatment cycle. Pregnancy test was performed 14 days after embryo transfer, and ultrasound scanning was done to check for the number of embryonic sacs and fetal heartbeat in 7 weeks. Clinical pregnancy referred to those cases where fetal heartbeat was detected.

This study was performed with patients' consent, and was approved by the ethical committee of the First Affiliated Hospital of SUN Yat-sen University.

Statistical analysis

The χ^2 test was performed using software Statistical Program for Social Sciences (SPSS)10.0. P value<0.05 was considered statistically significant.

Results

From April 2005 to January 2007, meiotic spindles of oocytes from 15 patients with male factor infertility requiring ICSI were assessed with Polscope. Maternal age was 31.6 ± 2.97 years. A total of 167 oocytes was retrieved (11.13 ± 2.75 per patient), and 80.2% of them were mature (9.07 ± 3.13 per patient), a total of 134 *in vivo* mature oocytes was used in this study. A control group of 15 couples treated for the same cause of infertility (mean maternal age= 34.1 ± 2.61 years) was included, 193 oocytes were collected (12.87 ± 2.95 per patient), of which 83.9% were mature (a total of 162 mature oocytes, 10.8 ± 2.43 per patient). No statistical difference was observed between the two groups in terms of maternal age, mean number of oocytes retrieved, and number of mature oocytes obtained.

Table 1 The spindle positions and the rates of fertilization, on-surviving, cleavage and top quality embryos of in vivo matured oocytes

Group	Number of oocytes (%)	Fertilization (%)	Non-surviving oocyte	Cleavage (%)	Top quality embryos (%)
I	30 (22.4)	28 (93.3)*	2	28 (100)	22 (78.6)
II	74 (55.2)	54 (73.0)	2	52 (96.3)	40 (76.9)
III	4 (3.0)	2	0	0	0
IV	4 (3.0)	1	0	1	0
V	22 (16.4)	14 (63.6)	3	13 (92.9)	8 (61.5)

* $P<0.05$, group I compared to other groups

In the IVM group, maternal age was 31.09 ± 1.13 years, 147 immature oocytes were collected (mean= 13.36 ± 1.18 per patient), 105 of these matured in vitro (mean= 9.55 ± 0.87 per patient). The percentage of total oocytes that matured to metaphase II was 71.4% (105:147). Meiotic spindles were observed at the time of ICSI.

In in vivo matured oocytes, the meiotic spindles were visualized in 83.6% of the oocytes (Table 1). The oocytes with the spindle directly underneath or adjacent to the first polar body (group I) had significantly higher fertilization rate compared to all other groups (group I versus II, $P=0.021$; group I versus III, $P=0.012$; group I versus IV, $P=0.0000$; group I versus V, $P=0.007$).

There was no statistical difference in the rate of non-surviving after ICSI between oocytes with or without spindle visible ($P>0.05$). The rate of cleavage was not significantly different among the groups ($P>0.05$). The rate of high quality embryos was also not significantly different among the groups ($P>0.05$). Single pronucleus was observed in three oocytes, one in group I and two in group II. All other oocytes were fertilized normally.

The spindle was visible in 77.1% of the in vitro matured oocytes (Table 2). Fertilization rate of oocytes in group I was similar compared to that of group II ($P>0.05$), but it

Table 2 The spindle positions and the rates of fertilization, non-surviving, cleavage and top quality embryos of in vitro matured oocytes

Group	Number of oocytes (%)	Fertilization (%)	Non-surviving oocyte	Cleavage (%)	Top quality embryos (%)
I	20 (19.0)	18 (90)	1	17 (94.4)	13 (76.5)
II	48 (45.7)	39 (81.3)	2	38 (97.4)	26 (68.4)
III	6 (5.7)	3 (50)*	1	3 (100)	2 (66.7)
IV	7 (6.7)	3 (42.9)*	1	3 (100)	1 (33.3)
V	24 (22.9)	12 (50)*	1	9 (75.0)	4(44.4)

* $P<0.05$, group III, IV and V compared to group I

Table 3 Distribution of the location of spindle relative to the first polar body in in vivo and in vitro matured oocytes

Group	Number of oocytes (%)		
	In vivo matured	In vitro matured	<i>P</i> value
I	30 (22.4)	20 (19.0)	0.529
II	74 (55.2)	48 (45.7)	0.144
III	4 (3.0)	6 (5.7)	0.296
V	4 (3.0)	7 (6.7)	0.178
V	22 (16.4)	24 (22.9)	0.210

was significantly higher than oocytes in groups III, IV and V (group I versus III, $P=0.029$; group I versus IV, $P=0.01$; group I versus V, $P=0.005$). However, the rates of cleavage and top quality embryos showed no significant difference among all groups ($P>0.05$).

The spindle positions of in vivo and in vitro matured oocytes are summarized in Table 3. There was no significant difference among all groups in terms of degree of displacement of the spindle ($P>0.05$).

The number of oocytes retrieved, the rate of fertilization, non-surviving, cleavage, top quality embryos, pregnancy and implantation of in vivo matured oocytes were compared with those of the control group without Polscope during ICSI. There was no statistical difference between the two groups. (Table 4)

There were a twin miscarriage, four live births and one ongoing pregnancy in the study group, whereas in the control group, four gave live births and two with ongoing pregnancies.

Discussion

Recent observations made with the use of Polscope have shown that the first polar body position does not predict accurately the location of the meiotic spindle in metaphase

Table 4 Results of the study group and the control group of in vivo matured oocytes

	Study group	Control	<i>P</i> value
Number of oocytes	134	162	
Fertilization (%)	99:134 (73.9)	130:162 (80.2)	0.19
Non-surviving oocyte (%)	7:134 (5.2)	9:162 (5.6)	0.9
Cleavage (%)	94:99 (94.9)	124:130 (95.4)	0.88
Top quality embryos (%)	70:94 (74.5)	80:124 (64.5)	0.12
Clinical pregnancy/ET(%)	6:15 (40)	6:15 (40)	1.0
Implantation rate (%)	7:32 (21.9)	7:35 (20)	0.85

II mammalian oocytes [2–4]. In these studies, meiotic spindles could be detected in 61.4–91.0% of oocytes, of these oocytes, 18.7–52.5% showed a minimal angle ($0\text{--}5^\circ$) of the meiotic spindle with regard to the polar body position. In our study, 22.4% in vivo matured and 19.0% of in vitro matured oocytes had their spindles close to the first polar body. Also, no spindle was detected in 16.4 and 22.9% of in vivo and in vitro matured oocytes respectively. This is in agreement with other studies [2, 3, 7, 8]. It is suggested that the first polar body is displaced from the spindle during manipulations for removal of cumulus and corona, as better alignment was seen when they were first observed [9]. To investigate whether mechanical manipulations did influence the positions of the spindle and the first polar body, we performed a study with 94 immature oocytes obtained in ICSI treatment cycles. They were matured in vitro (first polar body extruded), marking the position of the first polar body with a hole on the zona pellucida generated with laser, and observed with Polscope. Half of the oocytes were aspirated with a fine Pasteur pipette of a size slightly smaller than the oocyte for about 20 times to simulate the process of denuding, whereas the others were not manipulated and acted as the control group. The positions of the first polar body remained unchanged over the 72-h observation period, but the spindles moved in some of the oocytes in both the study and control groups (30.0 and 22.2% respectively). The spindles were not detected in 60% of the oocytes after 48 h, and increased to 70% in 72 h. Most of them stayed at the original location before disappearance (unpublished data). This shows that mechanical manipulations during denuding do not affect the positions of the spindle and the first polar body. Only a small proportion of spindles shifted after extended culture, while the others remained steady. This indicates that there might be more factors contributing to the dislocation of the spindles other than extended culture.

The spindle positions of in vivo and in vitro matured oocytes did not differ in our study. This is in conflict with findings of Moon [5], probably due to the different properties of human and mouse oocytes. Moreover, the immature oocytes used in this study did not go through maturation completely in vitro. In addition to the position of spindles, their morphology is also an important indication. This is not investigated due to limitation of resources, therefore it cannot be concluded that the spindles of in vivo matured oocytes are invariable to those of in vitro matured oocytes.

In this study, fertilization rate was significantly higher in in vivo matured oocytes with spindle close to the first polar body, and significantly lower in both in vivo and in vitro matured oocytes with no visible spindle. This is deviated from the findings of Rienzi [4], which states that fertilization rate is decreased only when the degree

between the spindle and the first polar body exceeds 90° , with increased abnormal fertilization. On the other hand, the rates of cleavage did not seem to relate to spindle locations, and the rate of good quality embryos showed a tendency to decrease from group I to group V, but not significantly. These findings suggest that spindle position may be not a good indicator of embryonic development, and spindle dislocation may be caused by more than one single reason.

It is believed that with the aid of Polscope, the spindle can be avoided during ICSI, thus enhancing fertilization and embryonic developmental potential [10]. However, in our study, the use of Polscope did not improve the rates of fertilization, cleavage, high quality embryo and pregnancy, while non-surviving was not significantly higher in the control oocytes, which were injected without Polscope. The main reason is that although the first polar body does not indicate exactly the spindle position, it is unusual that they are far apart. In in vivo matured oocytes, 77.6% had the angle of between the spindles and the first polar body less than 45° , and only 3% exceeded 90° . Therefore if the injection entry site is at the opposite hemisphere of the oocyte during ICSI, the spindle is less likely to be damaged, thus decreasing the rate of non-surviving.

Conclusions

The relationship between spindle location and embryonic development of in vivo and in vitro matured human oocytes was investigated in this study. In vivo matured human oocytes showed similar spindle positions compared to in vitro matured oocytes. Meiotic spindle location appears to influence fertilization rate, which was significantly higher in in vivo matured oocytes with spindle close to the first polar body, and significantly lower in both in vivo and in vitro matured oocytes with no visible spindle. The use of Polscope did not improve the results of ICSI in this study, so it may not be necessary purely for ICSI.

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