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Original Research Article

The spindle of oocytes observed by polarized light microscope can predict embryo quality

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ABSTRACT

Background: The aim is to evaluate spindle position of metaphase II oocyte and the development of embryos originated from oocytes with spindle and without spindle.

Methods: Cross-sectional analysis Research: 250 MII oocytes were analyzed with polarized microscope in Military Institute of Clinical Embryology and Histology, Vietnam Military Medical University.

Results: Spindles were detected in 170 (77.98%) of 218 metaphase II oocytes, 115 spindles (67.65%) of MII oocytes is beneath or adjacent to the first polar body, 55 oocytes had the spindle located between 300 and 1800 away from the first polar body. Fertilization rate and the rate of good quality embryos in oocytes with a visible spindle (77.98% and 61.02%) were higher than those in oocytes without a visible spindle (22.02% and 36.84%), the difference was statistically significant with $p < 0.001$ and $p < 0.05$.

Conclusions: The spindle position of metaphase II oocytes is not always beneath or adjacent to the first polar body. Fertilization rate and the rate of good quality embryos in oocytes with a visible spindle were higher than those in oocytes without a visible spindle.

Keywords: ICSI (intracytoplasmic sperm injection), Metaphase II oocyte, Polscope, Spindle

INTRODUCTION

Identifying whether an oocyte in metaphase II (MII) arrest or in the early phases of activation would be valuable for IVF, intracytoplasmic sperm injection (ICSI), and cloning, because preactivated oocytes cannot be fertilized normally.^{1,2} In addition, spindle dynamics during the initial phases of activation in living oocytes could provide a new marker for embryo quality.

Besides inducing biochemical alteration, oocyte activation is associated with several morphologic changes, including cortical granule exocytosis, reorganization of the fine structure of the zona pellucida, extrusion of the second polar body, and pronuclear formation.¹⁻³ However, most of these morphological changes are difficult to observe without fixation,

immunostaining, and fluorescence microscopy. Spindle movements or subtle changes in the meiotic spindle occurring in living oocytes might not be observed by the light microscopy.

A method for identifying whether an oocyte is in MII arrest or transition to anaphase or telophase II would allow for prompt decisions regarding whether artificial activation of oocytes would be required after ICSI.

Failure of oocyte activation has been one of the main causes of fertilization failure after ICSI, and subsequent or combined activation treatment has improved fertilization.^{4,5}

Moreover, the abnormal genetic oocytes are made by ovarian stimulation with impossibility in passing the

physical limitation that can cause failure of embryos development inside uterus. Oocyte selection with best quality without genetic abnormalities by non-invasive technique is essential.

Polarized light microscopy images birefringence, an optical property that result from molecular order and allows visualization of highly orderal biological structures such as spindles.⁶⁻⁹

Polarized light microscope allows embryologist to obverse morphology of the spindle. The appearance of spindle helps us recognize either oocyte maturation or the quality of laboratory.

There are two goals in this research:

- Evaluation of spindle location of mature oocytes
- Evaluation of embryos development by oocytes with spindle and oocytes with not-observed spindle.

METHODS

Clinical samples

During the period covering January to March 2018, mature oocytes of IVF patient at Military Institute of Clinical Embryology and Histology, Military Medical University, Hanoi, Vietnam.

Selection criteria: patients under 35 years old, 3ng/ml < AMH < 10ng/ml, the number of secondary follicles of 5-15 on the second or third day of menses cycle; the absence of polycystic ovaries, low response to ovary stimulation, low quantity and quality sperm.

Equipments and materials

POLARIZED LIGHT MICROSCOPE Ti-U Nikon TE2000 with Module polarAIDE – Octax laser system, Digital Camera. Cultural media of Vitrolife (Sweden). Bench top incubators (Cook)

Methods

Cross-sectional analysis Research

Oocyte preparation

The controlled ovarian stimulation, following the GnRH antagonist protocol. Ovary was under ultrasound daily until having at least 3 follicles bigger than 17mm. Ovulation was triggered by hCG (human Chorionic Gonadotropin) 5000IU injection.

Retrieve Oocytes after injection hCG 35-36h. After retrieving, put oocytes in incubator at 37oC, 5% CO2 for 2 hours.

Then denude Oocytes by enzyme hyaluronidase, put fresh Oocytes in incubator for 1 hour. Evaluate maturation level of oocytes and spindle before ICSI.

Cultures

After ICSI 16-18h, put zygote into new medium (G1 plus). Evaluate Embryos in the second day, and then put them into G2 plus in Incubator Cook Classify Embryos in the third day basing on criteria of Alpha of ESHRE 2011.¹⁰

RESULTS

Following table 1 in 218 matured oocytes, there are 170 visible spindle oocytes (77,98%) and 48 not observed spindle oocytes (22,02%).

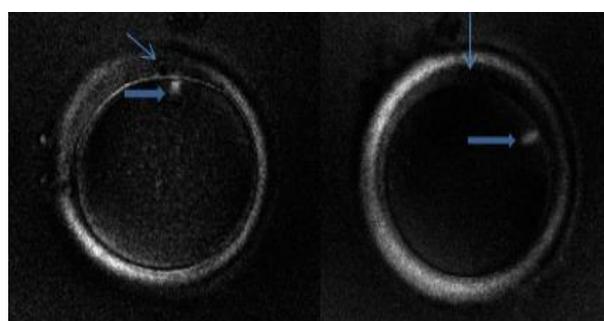


Figure 1: MII observed by polarized microscope, with magnification x200

The result of table 2 showed that of 170 visible spindle matured oocytes, there were 115 oocytes (67,65%) with spindle directly beneath and adjacent to the first polar body, 40 oocytes (23,53%) with spindle located between 30 and 60 degrees away from the first polar body, 10 oocytes (5,88%) with spindle located between 60 and 120 degrees angle, 5 oocytes (2,94%) with spindle located between 120 and 180 degrees angle. (The thin arrow: the first polar body).

The bold arrow: meiosis spindle, beneath the first polar body, the right image shows that the spindle located in 70 degrees way from the first polar body).

The fertilization rate of Mature oocytes with visible spindle was 69,41%, whereas this of mature oocytes with not-observed spindle was 39,58%. The difference was statistically significant with p <0,001.

Table 1: The rate of visible spindle in the mature oocytes.

MII	MII with visible spindle	MII not observed spindle
218 (100%)	170 (77,98%)	48 (22,02%)

Table 2: Spindle location.

MII with visible spindle	Directly beneath and adjacent to the first polar body	30-60 degrees	60-120 degrees	120-180 degrees
170 (100%)	115 (67.65%)	40 (23.53%)	10 (5.88%)	5 (2.94%)

The rate of good embryos among MII oocytes with visible spindle was higher than that among MII oocytes

with not-observed spindle (61,02% and 36,84%). The difference was statistically significant with $p < 0,05$ (table 3).

Table 3. The preclinical characteristics.

Classification	MII oocytes with visible spindle	MII oocytes not observed spindle	p
The fertilization rate	69.41% (118/170)	39.58% (19/48)	< 0.001
Embryo classification			
Good	61.02% (72/118)	36.84% (7/19)	< 0.05
Fair	27.12% (32/118)	47.37% (9/19)	> 0.05
Bad	11.86% (14/118)	15.79% (3/19)	> 0.05

DISCUSSION

In this research, oocytes were observed at 39 hours after hCG administration, which is the time that Cohen et al. 2004 had observed the spindle of MII oocytes with the highest rate (81.5%).¹¹

In this research, in spite of 48 oocytes with the first polar body, the rate of invisible spindle oocytes was 22,02%. To explain the absence spindle in MII oocytes, Montag et Al using time-lapse to observe the oocyte maturation in the in-vitro showed that when MI oocytes extruded the first polar body, a spindle had established the bridge between the polar body and the oolemma for 75-90 minutes. These imagine was recorded at Telophase 1 by polarized microscope. After extruding the first polar body, it took 115-150 minutes to establish the spindle beneath the first polar body. Therefore, the spindle absence was in the oocyte meiosis.¹²

In ICSI, the spindle location of mature oocyte was involved to the first polar body. The ICSI micropipette had to avoid the spindle in the injection. However, several researches reported that the first polar body did not exactly predict the spindle location.¹³ Thus the spindle could be injured when located far from the first polar body.

The use of polarized microscope could reduce spindle injured in ICSI. In this research, there were 8 oocytes (8,7%) with spindle located between 600 and 1200 degrees away the first polar body, this location was for injecting by the ICSI micropipette though out the 3-hour position in the oocyte. To explain the change of spindle location away from the first polar body, in the oocyte denudation by physical processing, the first polar body

was moved. Another reason was that the fluid surrounds the spindle forced its movement.¹⁴

Oocyte assessment by using polarization microscope improved the prediction of oocyte quality. In the Woodward's research 2008, the fertilization rate and embryo quality in day 3 and day 5 of the oocyte MII with visible spindle were higher and better than those of the oocyte MII with not-observed spindle.¹⁵ In our research, fertilization rates between two groups were different significantly statistically ($p < 0,001$). This result is similar to the result of Cohen et al, Heindryckx.^{11,16}

There are many reasons for the IVF failure, in which the major reason is from chromosome abnormalities and 80% of aneuploidy embryos are from the oocyte meiosis 1.¹⁷ Chromosome dividing errors often happen in meiosis 1. The main mechanism is the error in homologous chromosome pairing in Prophase 1, recombination and early dividing of chromosome. Spindle controls the chromosome movement though many periods in meiosis and has a crucial role of meiosis.¹¹

According to Moon et. al. 2003 implemented in 626 MII oocyte, the rate of good embryos among MII oocytes with visible spindle 64,2% was higher than that among MII oocytes with not-observed spindle 35,9% ($p < 0,01$), and the rate of multi-pronuclear in MII oocytes with visible spindle (3PN, 4PN) was reduced, compared to MII oocytes with not-observed spindle (5,9% and 10,7%).¹⁴

CONCLUSION

The spindle location may be far away from the first polar body. The fertilization and good embryo rates of MII

oocytes with visible spindle were higher than those of MII oocytes with not-observed spindle.

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