Polarization properties of mouse oocyte captured by Mueller matrix imaging

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Abstract. Non-invasive assessment of oocyte quality is of important basis in embryo transfer and other related fields. Until now, the oocyte quality assessment is mainly focus on the morphology observation. Oocyte quality is often reflected in varieties of its microstructure which can be provided by polarization imaging technology. The Mueller matrix is a label-free technique to characterize all polarization properties of samples. This paper used a Mueller matrix microscope for the first time for non-invasive imaging the mouse oocyte. By Mueller matrix analysis, we successfully examined the dichroism, phase delay and scattering depolarization of oocytes, furthermore used the above to compare the polarization properties of mouse oocytes at GV stage and MII stage and the polarization properties of matured fresh oocytes and postovulatory aged oocytes. In this paper changed phase delay were observed before and after oocyte maturation (and oocyte aging). It suggested that the Mueller matrix imaging may provide a potential breakthrough for oocyte quality assessment.

Keywords. Mueller matrix imaging, mouse oocyte, polarization

1. Introduction

As female germ cell, oocytes are the key raw material for embryo engineering and in-vitro fertilization, and play important roles in human assistant reproduction and animal reproduction. The oocyte quality is the decisive factor affecting the embryo development [1, 2]. The impaired oocyte quality can reduce the developmental competence of embryo, and increase the rates of abortion, stillbirth and birth defect [3-5]. Therefore, collecting high-quality oocytes has important reality significance for human assistant reproduction and animal embryo production.

In practice, an intuitive method is established to evaluate the oocyte quality based on its morphological detection [6]. For example, the morphologies of zona pellucida, cytoplasm and perivitelline space in oocytes were examined using the ordinary microscope [7, 8], or the spindle morphology was examined using LC-polscope system [9]. This kind of method of morphological detection has the common advantage of non-invasive intervention, namely, optical detection causes little damage to the oocyte development potential. It is widely used in practice. However, this evaluation system based on morphology identification also presents the characteristic of subjectivity (depending more on the experience of technicians), which increases the error of oocyte quality evaluation.

Many researches have reported that the components and structure in the diseased tissue would change [10-12]. Subsequently, the optical properties changed accordingly affecting the imaging
intensity, absorption spectra, transmission spectra, scattering spectra, and so on [13-15]. It is known that light is a transverse electromagnetic wave. When the tissue alters pathologically, the polarized state of the emitted light from the tissue would also change relative to the incident light. One of the applications is that the polarization imaging can identify the cancer tissue [16, 17].

Mueller matrix is a $4 \times 4$ matrix which records the change of polarization state between the incident and emitted light of sample. All the polarization information of the sample can be extracted from its Mueller matrix. So far, although Mueller matrix elements have not been found the clear physical meaning, but the polarization information of dichroism, phase delay and scattering depolarization can be obtained after decomposing or transforming Mueller matrix [18-20]. Therefore, Mueller matrix shows the outstanding application and research value among the numerous polarization detection technologies.

In this paper, the Mueller matrix of mouse oocyte was firstly acquired using a self-rebuilt Mueller matrix microscope, further we also compared the polarization properties of mouse oocytes at different development stage and the polarization properties of postovulatory oocytes before and after aging.

2. Materials and methods

2.1. Oocyte collection
The ovaries from the Kunming white female mice aged 7-8 weeks, which were euthanized by cervical dislocation, were removed and transferred to human tubular fluid medium, and then were punctured with sterile needle to release denuded GV oocytes for the research.

Kunming white female mice aged 7-8 weeks were intraperitoneally injected with Pregnant Mare Serum Gonadotropin 10 IU, and were injected with human chorionic gonadotrophin (HCG) 10 IU 48 hours later. After 15 hours, their ovary and fallopian tube were removed aseptically. After removing the adipose tissue, the ovary and fallopian tube were separated. The sterile needle tore the oviduct bulge, and then a mass of oocytes packed by granular cells were released. After incubating in hyaluronidase for 5 minutes, the fresh matured oocytes which were at the meiosis II (MII) stage were collected. The postovulatory aged oocytes were established via in-vitro culturing the fresh matured oocytes for 10 hours.

2.2. Mueller matrix imaging
This paper transformed the Mueller matrix microscope from a commercial microscope. As shown in figure 1(a), the bottom illumination source had the central wavelength of 632.8 nm and the spectral width of 20 nm. A linear polarizer and a quarter-wave plate were inserted in front of the bottom illumination in turn, forming a polarization state generator. Similarly, a quarter-wave plate and a linear polarizer were inserted in front of a CCD camera in turn, forming a polarization state analyzer. Changing the orientation of the linear polarizer and the quarter-wave plate in the polarization state generator can constructed the incident light with six polarization states, including horizontal linear polarized light, vertical linear polarized light, 45° linear polarized light, 135° linear polarized light, left circularly polarized light and right circularly polarized light. Likewise, the polarization state of the light emitted from each incident light passing through the sample were measured according to the above six polarization states. Then, we can obtained the Mueller matrix of the sample.
Figure 1. Schematic diagram of Mueller matrix imaging system (a) and sample diagram (b).

2.3. Mueller matrix processing and operation

Mueller matrix is a coefficient matrix associating incident light with the emitted light, which contains all polarization information of the sample. According to the Arteaga’s work [21], the system errors were calibrated by measuring the Mueller matrix of air. In this work, the incident light is emitted after passing through the culture dish, oocyte and culture medium in turn (figure 1b), that is, the Mueller matrix of sample is the product of the Mueller matrix of culture dish, the Mueller matrix of oocyte and the Mueller matrix of culture medium, $M_{\text{sample}} = M_{\text{medium}} \cdot M_{\text{oocyte}} \cdot M_{\text{dish}}$. Thus, $M_{\text{oocyte}} = M_{\text{medium}}^{-1} \cdot M_{\text{sample}} \cdot M_{\text{dish}}^{-1}$. Subsequently, the Mueller matrix of oocyte was decomposed to obtain the polarization information with clear physical meaning, such as, the dichroism, phase delay and scattering depolarization of oocytes (the principle of matrix decomposition and the specific formula was cited in reference [19]).

2.4. Statistical analysis

Results are expressed as the means ± SEM. Statistical comparisons were performed using One-way ANOVA with software SPSS 16.0. $p<0.05$ is considered significant. The number of oocytes used for statistics was about 10 in each group.

3. Result and discussion

3.1. A typical application example of Mueller matrix and polarization properties of oocyte

This work firstly acquired the Mueller matrix images of mouse oocyte. Taking the mouse oocyte at GV stage as example, figure 2 shows the normalized Mueller matrix images of GV oocyte. After decomposing the Mueller matrix, the normalized images of dichroism, phase delay and scattering depolarization of GV oocytes were obtained (figure 3a-3c). This work defined the zona pellucida, cytoplasm and cytoplasm cortex as the characteristic regions of oocytes, and compared the values of the three parameters of dichroism, phase delay and scattering depolarization at these three regions (the values of the three parameters were represented by the normalized average gray value of the corresponding regions in the corresponding images). As shown in figure 3d, there was no significant difference in the dichroism (phase delay and scattering depolarization) value at the cytoplasm, zona pellucida and cortex of GV oocytes.
Figure 2. Mueller matrix of mouse GV oocyte, each matrix element was divided by m11 (namely performing normalization).

![Mueller matrix of mouse GV oocyte](image)

Figure 3. Images of dichroism, phase delay and scattering depolarization of mouse GV oocyte (a-c) and comparison of their values at the cytoplasm, zona pellucida and cytoplasm cortex (d), the representative position of which are labelled by rectangle 1, 2 and 3 in (a). Dotted circle indicates the Germinal vesicle.

![Images of dichroism, phase delay and scattering depolarization](image)

Table 1. Comparison of dichroism, phase delay and scattering depolarization at the cytoplasm, zona pellucida and cytoplasm cortex between the oocytes at GV stage and at MII stage.

|                     | Dichroism | Phase delay | Scattering depolarization |
|---------------------|-----------|-------------|---------------------------|
| **Cytoplasm at GV stage** | 0.13±0.03 | 0.54±0.07*  | 0.06±0.03                 |
| **Cytoplasm at MII stage** | 0.20±0.04 | 0.31±0.06   | 0.05±0.02                 |
| **Zona pellucida at GV stage** | 0.11±0.02 | 0.48±0.09   | 0.05±0.02                 |
| **Zona pellucida at MII stage** | 0.08±0.03 | 0.44±0.07   | 0.04±0.03                 |
| **Cortex at GV stage**     | 0.12±0.05 | 0.42±0.09   | 0.05±0.04                 |
| **Cortex at MII stage**    | 0.19±0.04 | 0.48±0.07   | 0.08±0.03                 |

* there is significant difference between the GV group and the MII group.

3.2. Comparison of polarization properties of oocytes at GV stage and at MII stage

Using the method described in Section 2.1 above, this work compared the dichroism, phase delay and scattering depolarization at the cytoplasm, zona pellucida and cytoplasm cortex between the oocytes at GV stage and the oocytes at MII stage. As shown in table 1, the phase delay value at the cytoplasm of GV oocytes was significantly higher than that of MII oocytes. The phase delay value of other regions (zona pellucida and cytoplasm cortex) had no significant difference between the GV and MII oocytes. Additionally, there was no significant differences in dichroism and scattering depolarization at each region between the GV oocytes and the MII oocytes. It is inferred that the parameter of the phase delay of mouse oocytes may change with the maturation process of oocytes, and the polarization properties of oocytes is expected to effectively and objectively evaluate the development quality of oocytes.
3.3. Comparison of polarization properties of fresh matured oocytes and postovulatory aged oocytes
In order to further confirm the correlation between the polarization properties of oocytes and their
development quality, this work used the above method to compare the polarization properties of fresh
matured oocytes and postovulatory aged oocytes. It can be seen (table 2) that the phase delay value
of cytoplasm in the fresh group was significantly lower than that in the aging group and the phase delay
value of zona pellucida and cytoplasm cortex in the fresh group had no differences from that in the
aging group. The dichroism and scattering depolarization of the cytoplasm, zona pellucida and
cytoplasmic cortex were not significantly different between the fresh and aging groups. The above
results suggested that postovulatory aging may affect the phase delay of mouse oocyte cytoplasm.

Table 2. Comparison of dichroism, phase delay and scattering depolarization at the cytoplasm, zona
pellucida and cytoplasm cortex between the fresh matured oocytes and the postovulatory aged oocytes.

|                                | Dichroism | Phase delay | Scattering depolarization |
|--------------------------------|-----------|-------------|---------------------------|
| **Cytoplasm in the fresh group** | 0.13±0.05 | 0.28±0.07*  | 0.04±0.02                 |
| **Cytoplasm in the aging group** | 0.17±0.06 | 0.50±0.06*  | 0.10±0.03                 |
| **Zona pellucida in the fresh group** | 0.09±0.03 | 0.43±0.08   | 0.02±0.02                 |
| **Zona pellucida in the aging group** | 0.11±0.04 | 0.43±0.07   | 0.05±0.03                 |
| **Cortex in the fresh group**    | 0.20±0.04 | 0.45±0.06   | 0.09±0.04                 |
| **Cortex in the aging group**    | 0.20±0.03 | 0.58±0.09   | 0.18±0.0                  |

* there is significant difference between the fresh group and the aging group.

4. Conclusion
It is the first time that Mueller matrix images of mouse oocytes were successfully acquired and the
polarization properties of different positions at oocytes were obtained. Then, this method was firstly
used to compare the polarization properties of oocytes at different development stages (GV stage and
MII stage) and with different quality (fresh matured oocyte and postovulatory aged oocyte), and the
differences in the phase delay parameter between the oocytes at different stage and with different
quality were observed. It can be seen that the polarization properties of oocyte are closely related to its
quality, and the parameter of phase delay is relatively high sensitivity to reflect the oocyte quality. (It
should be pointed out that more sensitive polarization parameters need to be further investigated in the
follow-up work.)

5. References
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