Measurement of pO$_2$ in cultured mouse oocytes using electron paramagnetic resonance oximetry

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ABSTRACT

We investigated the feasibility of measuring the partial pressure of oxygen (pO$_2$) in cultured mouse oocytes by electron paramagnetic resonance (EPR) oximetry. Approximately 15 pL (0.015 μL) of Ox063, an oxygen-sensing paramagnetic material, at a concentration of 500 mM was injected into the ooplasm of mouse oocytes. When one, five and 20 oocytes were used, the sample of 20 oocytes was sufficient to yield an effective EPR spectrum for determining the pO$_2$. The mean pO$_2$ levels in oocytes cultured under tensions of 5 and 20% O$_2$ were 60.2 and 153.1 mmHg, respectively. The present study indicates that it is possible to utilize EPR oximetry with Ox063 for the measurement of pO$_2$ in cultured oocytes.

Oxygen tensions in follicular fluid (4) and the female reproductive tract (3) are ≤ 40% of atmospheric oxygen. Therefore culture and handling of oocytes/embryos under the atmospheric level of oxygen (about 21% O$_2$) results in oxidative stress to them through the excessive generation of reactive oxygen species (ROS) (1, 5). Reduced oxygen tension (5% O$_2$) in the gas phase for assisted reproductive technology (ART) such as in vitro fertilization (2) and in vitro embryo culture (6) was reported to be beneficial to embryo development. However, we need to handle and manipulate oocytes/embryos repeatedly in air during ART procedures such as transferring oocytes/embryos between culture dishes and intracytoplasmic sperm injection. These procedures may increase the partial pressure of oxygen (pO$_2$) and the level of ROS in oocytes/embryos. Therefore a method for repetitive measurement of the pO$_2$ in oocytes/embryos during ART procedures could be useful not only for monitoring the culture and handling conditions but also for improving the outcome of ART through examining the relationship between pO$_2$ and the level of oxidative stress in oocytes/embryos.

To our knowledge, data on pO$_2$ of mammalian oocytes/embryos are limited. In the hamster, pO$_2$ levels in oocytes and 2- to 8-cell stage embryos recovered from the oviducts were measured using a microelectrode technique (12). However, there is no information on the pO$_2$ in mammalian oocytes/embryos cultured or manipulated in vitro. For measuring the pO$_2$ in tissues, several methods have been developed but most techniques are limited in their ability to analyze a single cell such as an oocyte (13). Among the several known oximetry techniques, a method based on electron paramagnetic resonance (EPR) called EPR oximetry is minimally invasive, accurate, and allows for repeated measurements, and so has been utilized in many tissues (7) and cell suspensions (11). EPR oximetry uses exogenous oxygen-sensing paramagnetic probes whose EPR line widths are broadened linearly with respect to the pO$_2$ levels in the vicinity. Thus, the measured spectral line width can be converted to the oxygen concentration using an appropriate standard curve.

Among oxygen-sensing probes, the triarylmethyl radical Ox063 is a biologically stable and nontoxic molecule, and cannot permeate the cell membrane.
In the present study, we therefore examined the feasibility of measurement of pO\textsubscript{2} in cultured mouse oocytes using EPR oximetry with Ox063 as a preliminary study for monitoring the changes of pO\textsubscript{2} during ART procedures.

In our first experiment, the EPR spectra of one, five and 20 oocytes were obtained to determine the number of oocytes required to accurately measure pO\textsubscript{2}. In our second experiment, the EPR spectra of oocytes cultured under 5% and 20% O\textsubscript{2} atmospheres were measured to evaluate the potential application of this technique to monitor changes in pO\textsubscript{2} in oocytes and embryos. Ovulated oocytes surrounded by cumulus cells were collected from the oviducts of female ICR mice (23 to 25 days old) that were induced to superovulate by intraperitoneal injection of 5 IU of eCG and 5 IU of hCG given 48 h apart. The oocytes were then denuded of their cumulus cells using 300 units/mL hyaluronidase (H-3506; Type 1-S from the bovine testes, Sigma-Aldrich) at 37°C for 5 min. All experiments were conducted in accordance with the Hokkaido University guidelines for the care and use of animals and with an approved animal protocol from the university.

Denuded oocytes with a first polar body (metaphase II oocytes) were transferred to a 3-μL droplet of HEPES-CZB medium (CZB medium supplemented with 5.56 mM D-glucose, 20 mM HEPES and 5 mM NaHCO\textsubscript{3}) (8) covered with paraffin oil. Ox063, an oxygen-sensing paramagnetic probe, was dissolved in sterile physiological saline to a final concentration of 500 mM. Microinjection pipettes with a 1-μm inner diameter and 3-μm outer diameter were fabricated and loaded with 2 μL of this Ox063 solution just prior to the intracytoplasmic injection of the paramagnetic probe. Approximately 15 μL of this solution was injected into the ooplasm of each oocyte using a previously described DNA microinjection procedure (10). After the injection of Ox063 solution, the oocytes were transferred through 10 washes with 50-μL drops of HEPES-CZB (experiment 1) or gas-equilibrated CZB medium (experiment 2) under paraffin oil. Approximately 1 h after the incubation under air (experiment 1) or...

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**Fig. 1** pO\textsubscript{2} calibration curve for Ox063. (A) Examples of electron paramagnetic resonance (EPR) spectra for Ox063 showing the changes that occur upon exposure to 0 and 160 mmHg of O\textsubscript{2}. (B) Calibration curve for Ox063 against premixed gases of known pO\textsubscript{2} showing linearity over the range of interest. Scale bar, 25 μT.

**Fig. 2** EPR spectra of mouse oocytes in culture. EPR spectra for (A) one, (B) five and (C) 20 oocytes. (D) Spectra obtained for 25 and 23 oocytes cultured under 5% O\textsubscript{2} and 20% O\textsubscript{2} in a gas atmosphere, respectively. The wave height represents the concentration of the oxygen-sensing probe. Scale bars, 50 μT.
in a humidified atmosphere of 5% O$_2$ (5% O$_2$, 5% CO$_2$ and 90% N$_2$) or 20% O$_2$ (5% CO$_2$ in air) at 37°C (experiment 2), the oocytes with a normal appearance were transferred into a gas-permeable Teflon tube (AWG-18; Penn & Nitto Co., Kanagawa, Japan) with approximately 5 μL of the medium. The tube was then placed inside a quartz EPR tube, and the pO$_2$ was measured. The EPR spectrum of Oxo63 was recorded at room temperature using an X-band EPR spectrometer (JES-RE; JEOL Ltd., Tokyo, Japan). Typical spectroscopic conditions were a frequency of 9.43 GHz, an applied magnetic field of 336 mT, an incident microwave power ranging from 0.6–0.8 mW, and a modulation amplitude (100 KHz) equal to 0.015 mT. A calibration curve using the first derivative peak-to-peak line width of the EPR signal of Oxo63 (Fig. 1A) versus the oxygen concentration was used for the determination of the pO$_2$ level (Fig. 1B).

In experiment 1, one, five and 20 oocytes injected with Oxo63 were separately incubated for 1 h in HEPES-CZB medium under air at 37°C. Oocytes with a normal appearance were then subjected to pO$_2$ measurements. The EPR spectrum of the single oocyte could not be detected (Fig. 2A). Although the EPR spectrum of the five oocytes was detectable (Fig. 2B), it was difficult to determine the pO$_2$ with accuracy using this spectrum due to the low signal-to-noise ratio. As shown in Fig. 2C, 20 oocytes proved to be sufficient for the quantitative evaluation of pO$_2$ based on the obtained EPR spectrum, which had a much improved signal-to-noise ratio.

In experiment 2, oocytes injected with Oxo63 were divided into two groups and incubated in CZB medium in a humidified atmosphere under 5% or 20% O$_2$ at 37°C. After 1 h of incubation, approximately 20 (range, 17 to 25) morphologically normal oocytes from each group were transferred to a Teflon tube, placed inside a quartz EPR tube and cultured for another 2 h under a distinct O$_2$ tension. After culturing, the quartz EPR tube was sealed with paraffin wax film (Parafilm; Alcan Packaging Co., Cedex, France) and pO$_2$ measurements were taken. The spectra obtained from oocytes cultured under the distinct O$_2$ tensions are shown in Fig. 2D. The pO$_2$ levels in the oocytes cultured under 5% and 20% O$_2$ were calculated to be 60.2 ± 2.6 and 153.1 ± 4.1 mmHg (mean ± SD of 3 replicates), respectively. There are no comparable data; however, the mean pO$_2$ (about 60 mmHg) in the present mouse oocytes cultured under 5% O$_2$ was similar to the value (about 50 mmHg) in hamster oocytes recovered from the oviduct (12) in which the pO$_2$ was reported to be about 40 mmHg (about 6% O$_2$) (3). The mean pO$_2$ (about 150 mmHg) in the present mouse oocytes cultured under 20% O$_2$ was also similar to the value (about 155 mmHg) in the medium equilibrated to the gas atmosphere of 20% O$_2$ (14). These results indicated that we could utilize EPR oximetry with Oxo63 for the measurement of pO$_2$ in cultured oocytes.

About 70% (133/186) of the oocytes maintained a normal morphology at 1 h after injection with Oxo63. This percentage was similar to that found previously for pronuclear stage mouse oocytes (75%) that had received DNA injections (10). Although Oxo63 is extensively derivatized to confer chemical stability and low toxicity in tissues (9), the cytotoxicity of this agent in oocytes remains to be determined in order to establish a protocol to allow repeated measurements of pO$_2$ in the same oocytes over several hours of incubation.

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REFERENCES


