—Mini Review—

Evaluating the Quality of Human Embryos with a Measurement of Oxygen Consumption by Scanning Electrochemical Microscopy

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Abstract: *Morphological evaluation has been widely used to evaluate embryo quality because it is non-invasive and useful in predicting pregnancy rate. However, morphological evaluations are subjective and categorization standards often vary between investigators. The respiration rate of embryos is a useful parameter for evaluating embryo quality. The scanning electrochemical microscopy (SECM) measuring system provides a non-invasive, simple, accurate, and consistent measurement of the respiration activity of human embryos. After morphological evaluation by Veeck's method, oxygen consumption by individual human embryos was quantified by SECM. Fundamentally, the maturation of mitochondria correlated with an increase in oxygen consumption during the development of embryos. The development of mitochondria may be an important factor in embryo quality, because mitochondria provide ATP for embryonic development by metabolism of nutrients in the cytoplasm. The respiration rates on the day 3 after in vitro fertilization (IVF) were measured and significant differences in oxygen consumption were registered even among embryos with the same morphological classification. There were no significant differences between the mean rates of oxygen consumption at each cleavage stage, however, there was considerable variation in respiration rate within embryos of the same morphological grade. The safety of SECM is assured as the embryos which were examined by SECM for oxygen consumption showed the same development levels as the control group. These results support the hypothesis that measuring embryonic respiration provides additional and valuable information about embryo quality.*

Key words: *Embryo quality, Oxygen consumption, Non-invasive evaluation*

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Introduction

Morphological evaluation has been widely used to evaluate embryo quality because it is non-invasive and useful in predicting pregnancy rates. However, morphological evaluations are subjective and categorization standards often vary among investigators. Therefore, more objective selection criteria are needed. Respiration is a useful parameter for evaluating embryo quality as it provides important information about metabolic activity. The scanning electrochemical microscopy (SECM) measuring system, which was introduced by Abe *et al.* [1], provides a non-invasive, simple, accurate, and consistent measurement of the respiration activity of single human embryos.

Scanning electrochemical microscopy is a technique in which the tip of a microelectrode is used to monitor the local distribution of electro-active species near the sample surface. The SECM measuring system has

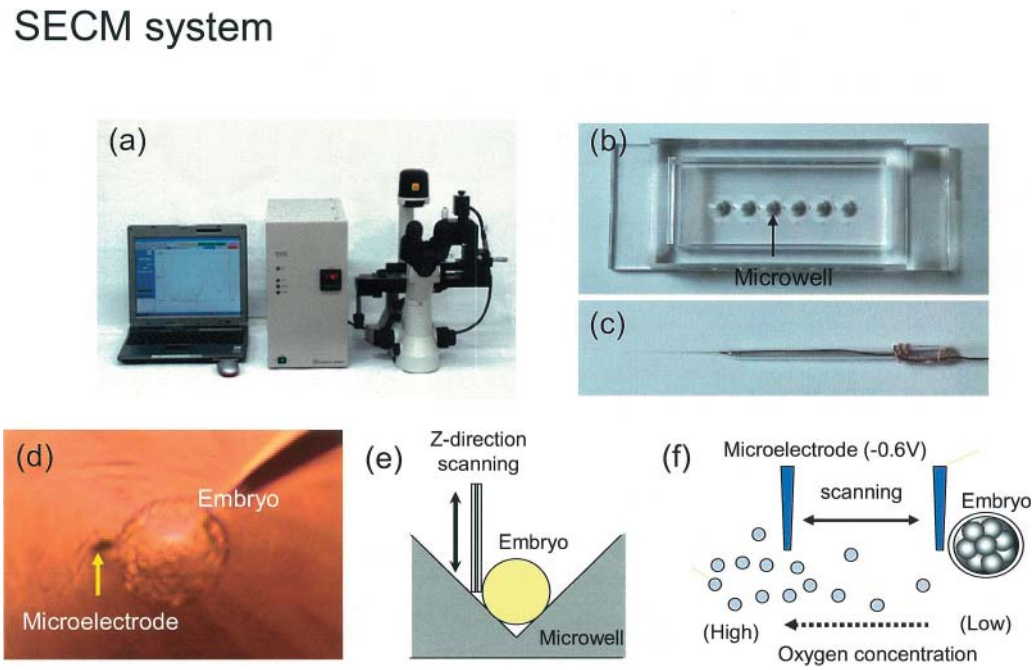

Fig. 1. The SECM system (a), a plate with microwells (b), and a microelectrode (c) for measurement of respiration activity of embryos. SECM includes a measurement instrument on the inverted optical microscope stage, a potentiostat, and notebook computer. The plate has six cone-shaped microwells (arrow). Individual embryos are transferred into a microwell filled with HFF99 medium. The sample sinks to the bottom of the well remaining at the lowest point (d, e). The oxygen concentration profiles are calculated with custom software based on spherical diffusion theory (f). Measurements of each embryo are performed very rapidly.

been used to non-invasively measure respiration activity of single bovine and murine embryos, among other species [2]. We employed SECM to accurately determine the oxygen consumption of single, identical human embryos at different developmental stages. In this article, we introduce the SECM method for assessing the quality of individual human embryos.

Measuring Respiration Activity of Single Human Embryo

Following *in vitro* fertilization (IVF)-embryo transfer procedure, surplus embryos that patients preferred not to keep preserved were used in our study. Informed consent for use of the embryos in this research was obtained from all the patients. From July 2006 to July 2007, 188 embryos from 73 cycles were examined. The mean age of the embryo donor was 34.5 ± 4.5 years and the number of previous ART cycles was 2.7 ± 2.3.

The embryos were cultured in Cleavage Medium (Sydney IVF, Australia) until day 3, after which they were cultured in Blastocyst Medium (Sydney IVF, Australia). After morphological evaluation by Veeck's method, oxygen consumption of individual human embryos was quantified with a modified SECM measuring system (Fig. 1). A single embryo was transferred into a well filled with HFF99 (Fuso Pharmaceutical Industries, Osaka, Japan) medium, where it fell to the bottom of the cone-shaped microwell and remained at the lowest point. A platinum (Pt)-microdisk electrode was lowered into the solution, and its tip potential was held at –0.6V vs Ag/AgCl with a potentiostat to monitor the local oxygen concentration. The microelectrode scanned along the *z*-axis from the edge of the sample and the oxygen consumption rate was calculated with custom software based on spherical diffusion theory. Measurements of each embryo were performed very rapidly (within 1 min). A part of each embryo was prepared for observation by transmission electron microscopy. The safety of SECM on the embryos was also examined.

The mean respiration rates ($F \times 10^{14}$/mol·s^{-1}) of 4-cell, 5-cell, 6-cell, 7-cell, 8-cell, 9-cell, and 10-cell embryos were 0.34 ± 0.1 (n=8), 0.45 ± 0.2 (n=15), 0.37 ± 0.1

Table 1. Oxygen consumption rates at each cleavage stage

Cleavage stage	No. of embryos examined	Oxygen consumption ($F \times 10^{14}$/mol·s^{-1})
4-cell	8	0.34 ± 0.1
5-cell	15	0.45 ± 0.2
6-cell	39	0.37 ± 0.1
7-cell	51	0.39 ± 0.2
8-cell	50	0.40 ± 0.2
9-cell	12	0.40 ± 0.1
10-cell	12	0.50 ± 0.2

There were no significant differences in the mean rates of oxygen consumption at each cleavage stage.

(n=39), 0.39 ± 0.2 (n=51), 0.40 ± 0.2 (n=50), 0.41 ± 0.1 (n=12), and 0.50 ± 0.2 (n=12), respectively (Table 1). There were no significant differences between the mean respiration rates at each cleavage stage; however, there was considerable variation in respiration rate within embryos of the same morphological grade.

The relationship between the embryo morphology and oxygen consumption was examined (Fig. 2). Significantly different levels of oxygen consumption were registered even among embryos of the same morphological classification.

After measuring their respiration rates with SECM, embryos were cultured to examine their developmental capacity. Embryos with moderate respiration rates (more than 0.26 × 10^{14} /mol·s^{-1} and under 0.56 × 10^{14}/mol·s^{-1}) had a 65.8% chance of reaching the blastocyst stage. On the other hand, embryos with lower (under 0.25 × 10^{14}/mol·s^{-1}) and higher (more than 0.55 × 10^{14}/mol·s^{-1}) respiration rates had only a 39.0% chance of reaching the blastocyst stage (Fig. 3).

The safety of SECM is assured as the embryos which were examined by SECM for oxygen consumption showed the same levels of development as the control group (Fig. 4).

Future Application of SECM in Assisted Reproductive Technology

Finding embryos of the highest quality is an imperative for obtaining good results in ART. To obtain a good embryo, many reports have proposed new methods and findings. A report on the correlation between first polar body morphology and pregnancy rate suggested that preselection at a very early stage may be helpful in identifying a subgroup of preimplantation embryos with good prognosis to form

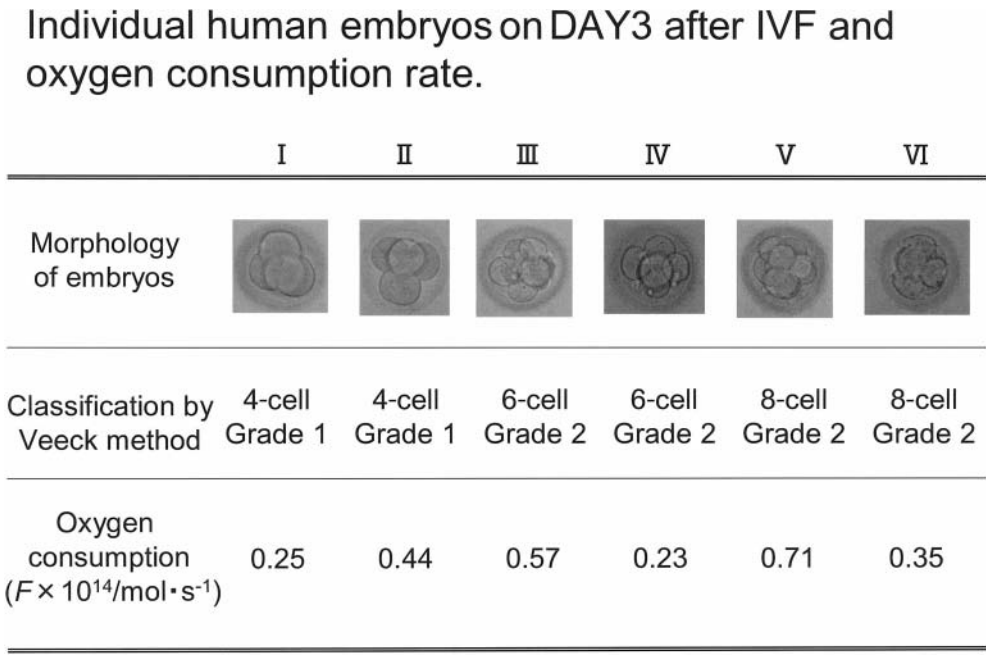

Fig. 2. Individual human embryos on Day 3 after IVF were classified by the method of Veeck *et al.* There were considerable variations in respiration rates within embryos (I and II, III and IV, V and VI) classified as the same morphological grade.

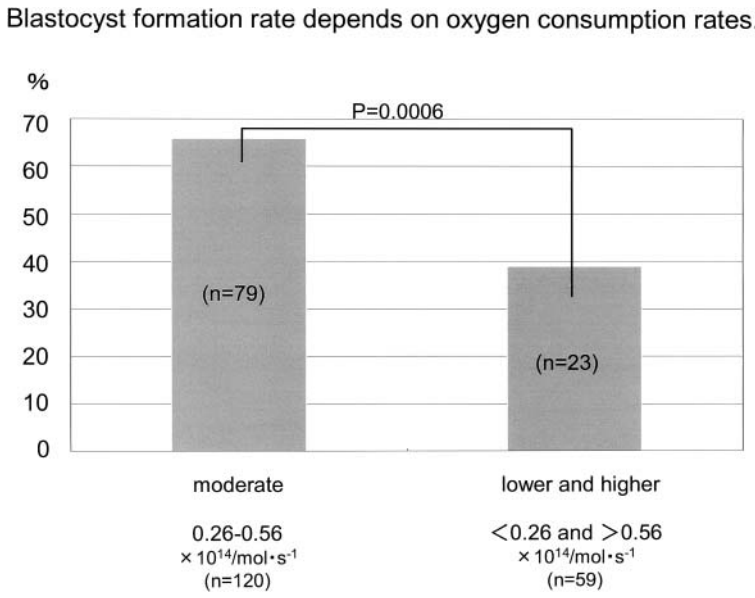

Fig. 3. Embryos with moderate respiration rates showed high developmental rate (65.8%) to the blastocyst. The developmental rate of embryos with lower and higher respiration rates was 39.0%.

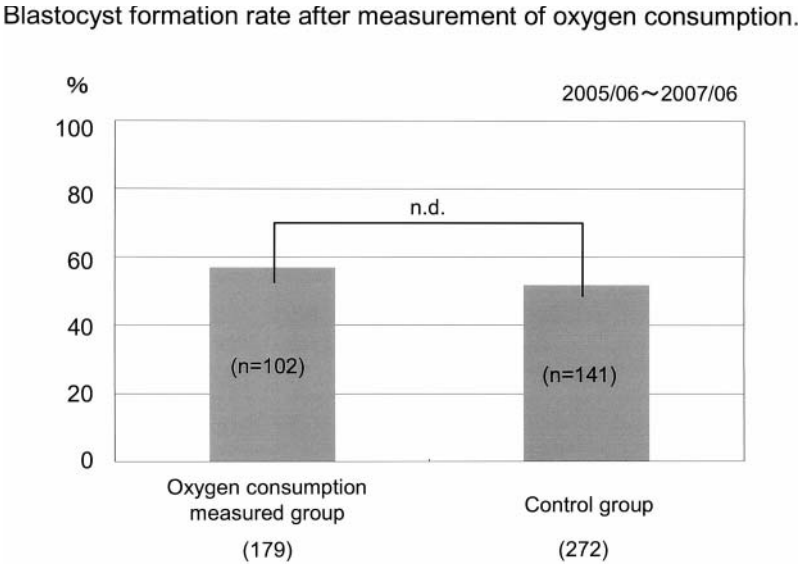

Fig. 4. The safety of SECM is assured as the embryos which were examined by SECM for oxygen consumption showed the same levels of development as the control group.

blastocysts and for implantation [3]. A report on the correlation between pronuclear pattern and pregnancy rate suggested that groups with particular pronuclear patterns tend to provide good results in pregnancy [4].

Morphological evaluation has been widely performed to assess embryo quality because it is non-invasive and useful in predicting pregnancy rate. Morphological observations have contributed significantly to the great success of ART programs in humans. In some cases, however, even embryos with low quality result in

successful pregnancy, whereas many good embryos, as judged by morphological observation, fail to result in pregnancy. Furthermore, morphological evaluation remains one of the most subjective and least quantitative aspects of embryo transfer, because categorization standards vary among the investigators. Recently, a very interesting study used time-lapse cinematography to morphologically analyze human embryonic development [5]. Numerous excellent findings by this method indicate that the dynamic observation of human gametes during the early stages of embryo development are of paramount interest for clarifying the physiological events during the fertilization process. The authors of that study suggested that there may be some limitations in momentary morphological observations of embryos which are dynamically changing [5].

The blastocyst stage transfer was proposed so that embryos could be evaluated, and some clinicians perform day 5 embryo transfer, even now. The principle idea is that, *in vivo*, cleavage stage embryos reside in the Fallopian tube and not in the uterus. The potential advantages of blastocyst culture and transfer include the synchronization of the embryo with the female tract leading to increased implantation rate and assessment of viability of an embryo before transfer [6]. The theory is sound and acceptable. The results of prospective randomized studies, however, clearly show that there is no advantage of the blastocyst stage transfer over the conventional cleavage stage transfer [7, 8].

Other new methods are needed in ART, especially selection methods to obtain the best quality embryos. Fundamentally, in embryos, the maturation of mitochondria is associated with increases in metabolism reflected by their oxygen consumption and CO_2 production [9]. So, mitochondrial function (oxygen consumption) may be an important parameter of embryo quality [9]. It is well known that metabolic processes change during embryonic development, as indicated by genome activation and large increases in protein synthesis. Oxygen consumption is a parameter used to gain valuable information on metabolic mechanisms. Oxygen consumption of mammalian embryos has been studied with various methods such as Cartesin a diver [9], spectrophotometrics [10–12], fluorescence [13–15] and electrochemical techniques [16–20].

The measurement of oxygen respiration rate (oxygen consumption) of bovine embryos has been employed to investigate the correlation between respiration rate and embryo morphology, diameter and sex, and it has been shown that the respiration rate is directly influenced by embryo diameter but does not differ between sexes [21]. So, respiration rates are only in partial agreement with embryo morphology, suggesting a slight discrepancy between these two methods of assessing embryo quality, and it is likely that a combined assessment of embryo respiration rate and morphology would improve embryo classification and subsequent selection.

The accuracy and simplicity of a measuring method is an essential and important point. With regard to this, the methods for measuring respiration rate for single bovine embryos were reported [22, 23]. The respiration activity of single bovine embryos entrapped in a cone-shaped microwell was monitored by scanning electrochemical microscopy. Using this method, the results for oxygen consumption rates of embryos of rank A (very good) were significantly higher than those of rank B (good). Furthermore, there were no apparent differences of oxygen consumption rate between male and female embryos [24]. These results indicate that the oxygen consumption rate of individual embryos reflects their quality but does not correlate with the sex ratio of embryos with excellent quality.

From the results of the present study, we deduce the following: 1) embryos have different oxygen consumption rates, even among morphologically similar embryos; 2) there is no correlation between morphological quality and respiration activity in human embryos at the early developmental stage; and 3) embryos with moderate respiration rates have better potential for further development than those with lower and higher respiration rates.

Conclusion

SECM can be used to measure the oxygen consumption of single human embryos at various developmental stages. The maturation of mitochondria correlates with the increase in oxygen consumption during the development of embryos. The development of mitochondria may be an important factor of embryo quality, because mitochondria provide ATP for embryonic development by metabolism of nutrients in the cytoplasm. The SECM technique may be a valuable tool for accurately assessing mitochondrial function and the quality of human embryos.

There was no correlation between morphological quality and respiration activity in human embryos at the early developmental stage. Embryos with moderate respiration rates had better potential for further

development than those with lower or higher respiration rates. The present results support the hypothesis that measuring embryonic respiration provides additional and valuable information about embryo quality.

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