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Title: Oxygen consumption measurement is a strong tool not only to elucidate the metabolism of blastocyst, but also to select suitable blastocyst for transfer

## Maezawa T<sup>1)</sup>, Yamanaka M<sup>2)</sup>, Hashimoto S<sup>2)</sup>, Fukuda A<sup>1)</sup>, Morimoto Y<sup>2)</sup> <sup>1)</sup>IVF Osaka Clinic, <sup>2)</sup>IVF Namba Clinic

**Objective:** Combination of morphology and oxygen consumption measurement has been reported to be effective for embryo selection. The present study was conducted to develop a novel method to assess the viability of blastocyst using mitochondrial oxidative phosphorylation (OXPHOS) indicated by oxygen consumption with FCCP:Carbonyl cyanide-p-trifluoromethoxyphenyl hydrazine (uncoupler) and cyanide (inhibitor of mitochondrial cytochrome oxidase).

Design: Prospective experimental study

**Materials and Methods:** Seventeen fresh BLs and 27 vitrified blastocysts were used after informed consent. The mean age of donors was 35.4 year-old. Vitrified blastocysts were used at 5 to 6 hours after warmed. Oxygen consumption of blastocyst cultured in HEPES-HTF at 37°C under 5%CO<sub>2</sub> in air was measured by a scanning electrochemical microscopy and used as a basic value. Oxygen consumptions were measured every 10 min for 40 min after addition of FCCP (1µM). Oxygen consumptions were measured again every 10 min for 30 min after addition of cyanide (1mM). The data are shown in a relative value to basic data. Moreover, Oxygen consumptions of vitrified-warmed shrunk blastocysts were compared each other.

**Results:** Oxygen consumptions of fresh and vitrified-warmed blastocysts in HEPES-HTF were 5.9 and 6.5 fmol/sec, respectively, and increased to a maximum value after an addition of FCCP (144% and 157%, respectively). The Oxygen consumptions dropped to 15.6% and 22.1% at 30 min after addition of cyanide, respectively. The basic Oxygen consumption of the shrunk blastocysts was 5.9 fmol/sec. However, some raised to 186.1% and the others remained to 102.2% after FCCP regardless of similar basic values as 5.6 fmol/sec and 6.4 fmol/sec, respectively. **Conclusions:** The present study suggests that 80-85% of Oxygen consumption depends on mitochondrial OXPHOS in human blastocyst. Vitrified-warmed blastocysts recovered their developmental potentials similar to fresh shown by Oxygen consumption. Suitable blastocyst for transfer could be selected from several shrunk blastocyst by measuring oxygen consumption. Oxygen consumption measurement

could be a useful tool for ART treatment.