

## RESPIRATION

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This paper will present different approaches to measure oxygen respiration rates of oocytes and the early stages of pre-embryo development. The outcome of these studies will be discussed outlining the progression of respiratory rates during embryo development, and the possible use of respiration rate measurements in selecting viable embryos. Finally, the oxygen consumption rates will be compared to the reservoir of substrates available to the embryo in the media.

**Introduction:** The metabolic uptake and release of specific compounds by individual human oocytes and pre-embryos is difficult to measure accurately due to tiny size (~ 0.1 mm) and biomass (~ 1 µg) of cleavage stage embryos, which is reflected in an almost undetectable mass flow to and from the embryo. Small incubation volumes (≤ 5 µl) and specialized media composition with a low background concentration of the analyte in question, as well as advanced analytical procedures are generally required to quantify the specific metabolic rates involved.

A notable exception is measurement of oxygen uptake (i.e. respiration) by cleavage stage embryos. Three particular properties combine to make respiration rate measurements possible: 1) substantial consumption rates as aerobic metabolism using oxygen as terminal electron acceptor is the most important energy generating process in oocytes and cleavage stage embryos; 2) low background concentration of oxygen in an aqueous media due to a low solubility of about 200 µmol/L at 37 °C; 3) high reactivity of oxygen, which makes it readily and specifically detectable by different microsensors and fluorescence indicators.

**Methods:** Two major approaches to measuring the oxygen consumption rates have been developed: A) closed respirometry based on oxygen depletion over time in micro vessels containing oocytes or pre-embryos. The oxygen depletion can be measured by change of buoyancy or fluorescence of an oxygen sensitive indicator within the closed vessel. B) open respirometry based on detection of steady-state concentration gradients of oxygen in the media surrounding the oocytes/pre-embryos. The latter approach can be automated with equipment that can be used in a clinical setting. However, the instruments are not yet approved for clinical use and are thus restricted to research applications.

**Results:** All experimental data indicate that the respiration rate of normal human oocytes and cleavage stage embryos is relatively constant around 4.5 to 6.5 fmol/s per embryo. From the morula stage to the blastocyst stage the respiration rate increases to 9.5 to 13.5 fmol/s in an expanding blastocyst.

Repeated measurements of respiration rates during development have revealed important details such as: i) a general correlation between respiration rate and cytoplasm volume for oocytes and cleavage stage embryos; ii) very low activity of the granulosa cells surrounding mature MII oocytes; iii) an increase in respiration rate by about 10% during oocyte maturation from GV to MII stage; iv) constant or slightly decreasing respiration rates during the first cell divisions after fertilization; v) slight temporary increases in respiration rates (3 to 10%) correlate with cytoplasmic cell division; vi) increasing respiration rates after activation of the embryonic genome in the compaction stage and further increase in respiration rate during blastocyst formation.

**Discussion:** Aberrant respiration rates for oocytes and cleavage stage embryos correlate with reduced viability (Abe et al, Kido et al). Higher oocyte respiration rates was found for developmentally competent oocytes (L.Scott et al.). Bovine studies have shown a correlation between an optimal respiration range for in vivo produced blastocysts and successful production of healthy offspring (Lopes et al.). While these studies and several others indicate a diagnostic potential, they also demonstrate the need for highly accurate rate measurements and preferably multiple rate assessments during development, and the need to correct the measured respiration rates for differences in embryo size. The clinical significance and applicability of these measurements still needs to be demonstrated in future studies.

**Interpretation:** The respiration rates of oocytes and pre-embryos are very small normally in the range from 5 to 10 fmol/s, where femto mol is  $10^{-15}$  mol. At this rate, it would take more than two months to use all the oxygen in an air bubble with a radius of 1 mm. This respiration rate is almost eleven orders of magnitude lower than the respiration rate of a grown adult. However, the difference in biomass is of a similar magnitude, so the oxygen consumption per weight for a developing embryo is almost identical to the average value for a resting adult (~13 µmol/g per hr).

Pyruvate uptake rates for cleavage stage embryos (Devreker et al.) are comparable to the measured oxygen consumption rates, i.e. 12 and 18 pmol/h per embryo, respectively. However, the relative rates may indicate that not all pyruvate is oxidized completely to carbon dioxide, as the stoichiometric conversion for complete oxidation of pyruvate is 1:2.5

A fertilized oocyte will use about 2.5 nmol of oxygen in total during its 5 day development from oocyte to expanded blastocyst. This is about half of the oxygen dissolved in a 20 µl media droplet. If 2.5 nmol of oxygen is used for aerobic respiration then it can completely oxidize about 0.08 µg of organic matter, which is about 1/8<sup>th</sup> of the organic content of the embryo itself. However, the oxygen consumption is low when compared to the carbon sources available in a media droplet. A 20 µl droplet of G1-PLUS media (VitroLife, Sweden) contain around 0.6 µg of pyruvate, 24 µg of organic substrates and 1000 µg of Human Serum Albumin (Gardner et al.). While the respiratory oxidation of carbon sources by the embryo during 5 days of development can substantially lower the pyruvate concentration by >10% , the potential effect on the total available organic substrates is <0,3%. If the media also include 5% w/w human serum albumin then the decrease in organic content due to embryo metabolism is even lower (<0,006%). It is thus not surprising, if it is difficult to detect an imprint on the general organic constituents of a growth media even after 5 days of incubation.

**References:** Abe (2007) *Journal of Mammalian Ova Research* 24:70; Devreker et al. (2000) *Fertil Steril* 73:947; Gardner et al. (2004) *Textbook of Assisted Reproductive Techniques 2<sup>nd</sup> ed.* p. 219; Kido et al. (2008) *Fertil Steril* 90:S341; Lopes et al. (2007) *Human Reproduction* 22:558; Scott et al. (2008) *RBMonline* 17: 461