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Relationships between oxygen consumption rate, viability, and subsequent development of *in vivo*–derived porcine embryos

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ABSTRACT

Oxygen consumption rate of *in vivo*–derived porcine embryos was measured, and its value as an objective method for the assessment of embryo quality was evaluated. Embryos were surgically collected 5 or 6 days after artificial insemination (AI), and oxygen consumption rate of embryos was measured using an embryo respirometer. The average oxygen consumption rate ($F \times 10^{14}$/mol s^{-1}) of the embryos that developed to the compacted morula stage on Day 5 (Day 0 = the day of artificial insemination) was 0.58 ± 0.03 (mean ± standard error of the mean). The Day-6 embryos had consumption rates of 0.56 ± 0.13, 0.87 ± 0.06, and 1.13 ± 0.07 at the early blastocyst, blastocyst, and expanded blastocyst stages, respectively, showing a gradual increase as the embryos developed. Just after collection, the average oxygen consumption rates of embryos that hatched and of those that did not hatch after culture were 0.60 ± 0.04 and 0.50 ± 0.04 for Day 5 ($P = 0.08$) and 1.05 ± 0.09 and 0.77 ± 0.05 for Day 6 ($P < 0.05$), respectively. The value and probability of discrimination by measuring the oxygen consumption rates of embryos to predict their hatching ability after culture were 0.56 and 63.6% for Day-5 embryos and 0.91 and 68.4% for Day-6 blastocysts, respectively. When Day-5 embryos were classified based on the oxygen consumption rate and then transferred non-surgically to recipient sows, three of the seven sows, to which embryos having a high oxygen consumption rate (≥0.59) were transferred, became pregnant and farrowed a total of 20 piglets. However, none of the four sows, to which embryos having low oxygen consumption rate (<0.59) were transferred, became pregnant. These results suggest that the viability of *in vivo*–derived porcine embryos and subsequent development can be estimated by measuring the oxygen consumption rate.

1. Introduction

An appropriate evaluation of embryo quality to select transferable embryos is important for the improvement of pregnancy rate after the embryo transfer. In general, embryo selection is usually carried out by observation of their morphology, relying on the subjectivity of the technician. On the other hand, several objective methods for evaluating embryos have been reported, such as by identifying viable cells [1] and measuring metabolic activities such as glucose intake [2], carbon dioxide generation, [3] and oxygen consumption rate [4–7]. However, in these studies, measurement of single embryos appeared to be

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difficult, and the treatments could potentially damage the embryos with harmful chemical exposures.

Because oxygen is consumed by oxidative phosphorylation and respiration in mitochondria and plays an important role in energy (ATP) production, respiration or oxygen consumption rate is considered as an appropriate index for the evaluation of embryo quality [8]. Recently, various methods have been reported for the measurement of mitochondrial oxygen consumption in embryos to evaluate their quality [8–14]. Moreover, high oxygen consumption rate of *in vivo*–derived bovine embryos was associated with a high conception rate after the embryo transfer [10,12,13]. However, there are only a few reports on the oxygen consumption measurement of porcine embryos [15–17] and/or on their subsequent developmental potential after the oxygen consumption measurements [15,16].

During the process of embryonic growth, mitochondria gain functional maturity, which is accompanied by a change in their morphology [18]. It has been reported that in bovine embryos, during the development from the morula to the blastocyst stage, mitochondria show a remarkable morphologic development highlighted by the expansion of cristae [18]. It is also clear that respiration activity changes corresponding to the development of mitochondria [18]. Although there are several reports about the differences in oxygen consumption rates between the developmental stages measured in single embryos [10–13,15–17], the optimum developmental stage for oxygen consumption measurement to assess embryo quality remained unclear. In cattle, the oxygen consumption rate remains low until the compacted morula stage and sharply increases thereafter until the expanded blastocyst stage [13,14]. Moreover, Shiku et al. [9] have reported that the hatching rate of *in vitro*–produced bovine morulae showing a high oxygen consumption rate ($>0.5 \times 10^{14}$/mol s^{-1}) was higher (68%) than that of morulae which consumed oxygen less than 0.5×10^{14}/mol s^{-1} (0%). Therefore, it is suggested that measurement of oxygen consumption rate at the morula stage is a possible way to predict developmental ability of embryos. However, in porcine embryos, the relationship between oxygen consumption rate of embryos and their subsequent developmental ability *in vitro* and *in vivo* remained unknown.

Our present study was designed to measure the oxygen consumption rate of *in vivo*–derived porcine embryos using an embryo respirometer and clarify the relationships between the oxygen consumption rate and the developmental stage, the cell number, and viability of the embryos. Pregnancy outcomes after transfer to recipients of embryos classified with “high” or “low” levels of their oxygen consumption rates were also investigated.

2. Materials and methods

2.1. Embryo collection from gilts

All animal-related procedures followed in this study were done with the approval of the Institutional Animal Experiment Committee of Kanagawa Prefectural Agriculture Facilities. Embryos were collected from 25 prepubertal gilts (Large White, Landrace, or Yorkshire) following the procedures described in our previous studies [15,19] with slight modifications. Superovulation was induced by an administration of eCG (Peamex 1500 IU intramuscular [im]; Sankyo, Tokyo, Japan) followed 72 hours later by an administration of hCG (Puberogen 500 IU im, Sankyo). The gilts were artificially inseminated twice, in the afternoon 1 day after hCG treatment and in the morning 2 days after hCG treatment. In the morning of Day 5 or 6 (Day 0 = the day of the first artificial insemination), the embryos were recovered from uterine horns by laparotomy under general anesthesia (4% to 5% [v:v] isoflurane) by flushing with a porcine oocyte/embryo collection medium [20] (POE-CM, Research Institute for the Functional Peptide [IFP], Yamagata, Japan). The embryos were kept in a porcine zygote medium (PZM-5, IFP) [21] under 5% CO_2, 5% O_2, and 90% N_2 at 38.5 °C until measurements.

2.2. Morphologic evaluation and measurement of embryo diameter

The collected embryos were classified by stages of development and quality standards of the Manual of the International Embryo Transfer Society [22], and only quality code 1 embryos were subjected to subsequent analyses. The diameters of embryos were measured using a digital camera for microscopes and attachment software (DXM1200 F and ACT-1; Nikon, Tokyo, Japan).

2.3. Measurement of the oxygen consumption rate

The oxygen consumption rate of the collected embryos was measured using an embryo respirometer (HV-405; IFP) according to the method of Abe et al. [14,18]. An embryo was placed at the bottom of the cone-shaped microwell on a respiration assay plate (RAP-1; IFP) filled with an embryo respiration assay medium (ERAM-2; IFP). A platinum microelectrode was set near the embryo, and a voltage of −0.6 V versus Ag/AgCl was applied to the microelectrode to reduce oxygen. The computer-controlled microelectrode scanned the *z*-axis (vertically) of the zona pellucida automatically at a moving speed of 30 μm/s for a distance of 160 μm. Each embryo was measured twice by scanning two different lines, and the oxygen consumption rate was calculated using analytical software developed for estimating the oxygen consumption rate based on spherical diffusion [9]. Measurement of individual oxygen consumption rate was completed within 1 minute.

2.4. Evaluation of cell numbers in embryos

Some embryos were subjected to differential staining of inner cell mass (ICM) and trophectoderm (TE) by the method of Thouas et al. [23]. Briefly, embryos were stained by incubating in PBS containing 0.2% (v:v) Triton X-100 and 100 μg/mL propidium iodide (P4170; Sigma Chemical, MO, USA) for 60 seconds and then stained and fixed by 25 μg/mL of bisbenzimide (Hoechst 33342; Calbiochem, CA, USA) in ethanol, at 4 °C in the dark for at least 3 hours. The stained embryos were rinsed with antifade solution (SlowFade S2828; Invitrogen, Carlsbad, CA, USA), mounted on a slide glass, covered with a cover glass, and observed under an