OXYGEN MEASUREMENTS AS INDICATORS OF CULTURE CONDITIONS AND EMBRYO VIABILITY

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Introduction
The aims of the present study were (1) to characterize oxygen and temperature conditions experienced by embryos during routine in vitro production (IVP) and (2) to evaluate oxygen consumption as a viability indicator for single embryos.

Materials and Methods
A combined oxygen-temperature microsensor (Fig. 1) was used to obtain oxygen partial pressure gradients and temperature profiles in either Exp. 1: 4-well dishes only with SOF medium (400 μl or 50 μl) or Exp. 2: day 3 or day 7 bovine IVP embryos (Holm et al. 1999, Theriogenology 52:683-700; Fig. 2).

In Exp. 1, measurements were performed 2 minutes after the dishes were taken from the incubator (5% CO₂, 5% O₂, 90% N). The 400 μl medium was measured from the top layer and ⅓ down into the well, whereas for the 50 μl droplets the profiles were obtained in the middle of the droplet.

In Exp. 2, embryos were evaluated morphologically and then loaded into one of the holes in a measuring block (Fig. 1), previously covered with 40 ml IVC medium and incubated for 3 days under 5% CO₂ in air (38.5°C, 100% relative humidity). Profiles were determined 75 minutes after removal from the incubator.

All measurements were performed under atmospheric air conditions at room temperature (24-25°C) (Exp. 1) or on a warming stage held at 37±1°C (Exp. 2).

Results

Experiment 1:
- In 400 μl wells (n=7), the oxygen partial pressure decreased with depth (21.0 ± 0.66% O₂ to 15.0 ± 1.6% O₂) while the average temperature increased from 27.2 ± 3.0°C to 31.7 ± 0.7°C (Fig. 3).
- In the middle of 50 μl droplets (n=5), the oxygen partial pressure was 17.1 ± 2.44% O₂ and the temperature 31.0 ± 1.11°C.

Experiment 2:
The average oxygen consumption was 0.25 ± 0.14 and 0.90 ± 0.56 nl/embryo/h for day 3 and day 7 embryos, respectively (Fig. 4).

No clear relation between respiration rate and embryo morphology could be demonstrated for day 3 embryos (data not shown).

For day 7 embryos, however, the average oxygen consumption was 1.17 ± 0.70, 0.98 ± 0.49 and 0.46 ± 0.38 nl/embryo/h for good, fair and poor quality day 7 embryos, respectively (Fig. 5).

Conclusions
(1) Routine handling of culture dishes outside the incubator influences temperature and oxygen conditions in the media surrounding the embryo.
(2) Respiration rate is lower in day 3 than in day 7 embryos.
(3) Oxygen consumption of day 7 embryos seems to be in agreement with their morphological quality.