Oxygen consumption of bovine granulosa cells *in vitro*

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Abstract

The oxygen consumption rate of granulosa cells is considered to be a key determinant of oocyte oxygenation in follicles. The oxygen status of the oocyte potentially dictates its developmental competence. However, quantitative information on the oxygen consumption rate of granulosa cells in literature is scarce. This limitation has hindered further investigation into the oocyte oxygenation, which could potentially be used as an indicator for selecting high quality oocytes for producing high quality embryos. This could ultimately contribute to improvement of the success rates of human In-Vitro Fertilisation.

In light of this issue, this work developed a method for measuring the oxygen consumption rate of granulosa cells *in vitro*. This included techniques related to granulosa cell harvest from cows, suspending/culturing granulosa cells in culture medium and development of a competent respirometer. Each measurement run on the oxygen consumption rate of granulosa cells was conducted by suspending the granulosa cell culture in the respirometer, in which an optical-based oxygen sensor probe was employed to continuously monitor the oxygen partial pressure change in the cell suspension.

Five separate sets of respirometer data were collected and used to calculate the oxygen consumption rate, giving a range of 2.1 to 3.3 x 10^{-16}mol.cell^{-1}.s^{-1}/0.16 to 0.25mol.m^{-3}.s^{-1}. These rates were comparable with but higher than other animal cell oxygen consumption rates reported by the literature. They were approximately 5 times higher than the oxygen consumption rate of granulosa cells harvested from sheep (Gosden & Byatt-Smith 1986).

The implications of the measured oxygen consumption rate were then examined in the context of oxygen transport in large bovine preantral follicles via an existing mathematical model. The resulting predicted oxygen profiles in large bovine follicles were consistent with the study of Redding *et al.* (2007), which showed that as a preantral follicle grew the oxygen transport across the follicle was increasingly strained, resulting in subsequent decrease in oocyte oxygenation. By applying the bovine specific parameter estimates to the model, this work predicted that the largest follicle radius for the oxygen transport in bovine preantral follicle was 134µm, beyond which the oxygen could not reach oocyte. Since the experimentally reported sizes of the large bovine preantral follicles ranged of 58 to 145µm in radius (see Section 7.1.2), this work proposed that the oxygen transport was capable of oxygenating the oocytes in all but the largest preantral follicles. If bovine preantral follicles were to grow larger than they are experimentally observed to do so, all oocytes contained within such follicles would be in a hypoxic state. This is a result consistent with other work.

Furthermore, based on the use of bovine and ovine specific parameter estimates in the model, this work found that oxygen transport in follicles was likely to be the result of a unique combination of parameters for a particular species. Specifically the oxygen consumption rate and fluid voidage of follicles of a given species would be the key determinants in oxygen levels across the follicle. This work also found that the fluid voidage range across large bovine preantral follicles was reasonably wide (0.34 to 0.65) and the value increases with the follicle size. This suggested that the use of fluid voidage for investigating the oxygen levels across

preantral follicles must be follicle size specific. Finally, this work presented a nomograph which described the relationship among fluid voidage, follicle radius and oxygen levels at oocyte surface. The nomograph can be used as a tool for further research to study the oxygen status of the oocyte during the growth of a large bovine preantral follicle and may be generalised to describe non-species specific follicle oxygenation in growing follicles.

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