Mathematical modelling of oxygen concentration in bovine and murine cumulus–oocyte complexes

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Abstract

Immature oocytes benefit from nutrient modification of the follicular environment by the surrounding cumulus mass. However, the oxygen concentration that the oocyte may be exposed to could be lower than the antral follicular concentration due to the metabolism of surrounding cumulus cells. Using metabolic data previously determined, we have developed a mathematical model of O₂ diffusion across the bovine and murine cumulus–oocyte complex. From this we have determined that across a physiological range of external pO₂, less than 0.25% and 0.5% O₂ is removed by cumulus cells within the bovine and murine cumulus–oocyte complex respectively. Our model differs from others as it: incorporates a term that allows for nonlinear variation of the oxygen consumption rate with oxygen concentration; considers two regions (oocyte and cumulus) sharing a common boundary, both of which consume oxygen at different non linear rates. Cumulus cells therefore remove little O₂, thus sparing this essential gas for the oocyte, which is dependent on ATP generation via oxidative phosphorylation.

Reproduction (2006) **131** 999–1006

Introduction

A quantitative understanding of nutrient supply to the oocyte in the antral follicle is generally lacking. There is good evidence that, initially, gap-junctional communication between the oocyte and the surrounding cumulus cells is important in nutrient transfer (Herlands & Shultz 1984), and the transfer of small molecular weight molecules, such as cyclic AMP (Bornslaeger & Schultz 1985), uridine (Herlands & Shultz 1984) and glucose (Saito *et al.* 1994) by this means has been described. Once the gap-junction connection has been severed in response to the ovulatory stimulus (*in vivo*) or spontaneous meiotic maturation (*in vitro*), the oocyte presumably relies on a histotrophic nutrient supply, although the close proximity to the expanding cumulus mass is likely to alter nutrient composition adjacent to the oocyte relative to that in the follicular fluid.

One component that appears to be important for the establishment of developmental competence in the oocyte within antral follicles is oxygen. In general, pO₂ levels around 7–11% have been recorded in antral follicles of humans and pigs (Knudsen *et al.* 1978, Fischer *et al.* 1992). Several studies in humans (Van Blerkom *et al.* 1997, Van Blerkom 1998) indicate that perifollicular vascularity is associated with oocyte developmental competence. This was subsequently

related to the dissolved O₂ content (Van Blerkom 1998), although this association has been disputed (Huey *et al.* 1999). In contrast, there are many studies that have examined the effect of O₂ concentration during *in vitro* maturation of oocytes across several species. Examples of such studies include hamster (Gwatkin & Haidri 1974), squirrel monkey (Yeoman *et al.* 1999), cow (Hashimoto *et al.* 2000, Minami *et al.* 2000), pig (Park *et al.* 2005) and mouse (Haidri *et al.* 1971, Eppig & Wigglesworth 1995, Hu *et al.* 2001). Such studies have not yielded any consensus over the optimal O₂ concentration during maturation, as there are conflicting results, especially in regard to the benefits of a low O₂ atmosphere. Of central importance to these studies is the choice of oxygen concentration used. Many have compared atmosphere (approximately 20% O₂) against 5% O₂ concentrations, the latter being the widely accepted optimal value for the development of mouse embryos *in vitro* (Quinn & Harlow 1978).

Recently we measured the uptake of key nutrients in bovine cumulus–oocyte complexes (COCs) to determine if oocyte-secreted factors influence bovine COC metabolism (Sutton *et al.* 2003). In contrast to the mouse (Sugiura *et al.* 2005), oocyte-secreted factors appear to have little influence over the metabolism of the surrounding cumulus cells. However, as with the mouse

(Sugiura *et al.* 2005), bovine COCs have significant levels of glycolysis, with lactic acid production accounting for much of the glucose taken up, especially prior to cumulus expansion (Sutton *et al.* 2003). These data suggest that oxygen consumption by the bovine cumulus cell mass is minimal, thus sparing O₂ for oxidative phosphorylation by the oocyte. We have therefore developed a mathematical model of the diffusion of oxygen through the COC so as to assess how the O₂ concentration decreases from the follicular-fluid cumulus oophorus interface to the corona radiata oocyte interface. We have applied this model to data already described for both bovine and murine COCs.

Materials and Methods

Cumulus–oocyte complex parameters

Data for COC diameter and O₂ consumption was obtained from a variety of published sources, identified in Table 1.

General assumptions

Prior to the luteinising hormone surge, cumulus cells are tightly packed around the oocyte and we have assumed that, in the case of passive diffusion of oxygen, these cumulus cells are effectively a homogeneous mixture of intra- and extracellular matter (Jones 1986). The diffusion coefficient of oxygen in the cumulus mass can therefore be taken to be constant. We have also assumed spherical symmetry of the COC, and ignored that at least part of it is attached to the mural granulosa cell layer of the follicle. The thickness of the zona pellucida (approximately 10 µm) has been neglected, as this is acellular

and does not consume oxygen, nor is it likely to impede oxygen diffusion. Finally, we have assumed that the follicular fluid surrounding the COC is perfectly mixed, enabling us to take the concentration of oxygen in this fluid to be constant.

Mathematical model

The approximate spherical symmetry of the COC and our interest in the very early stage of development justifies our use of a spherically symmetric steady-state model. The rate of oxygen consumption by both oocyte and cumulus is expected to vary with the concentration of oxygen. Thus we solve the equation

$$\frac{D}{r^2} \frac{d}{dr} \left(r^2 \frac{dC}{dr} \right) = M(C),$$

subject to suitable boundary conditions, for the oxygen concentration C as a function of radial position r from the centre of the COC, where D is the diffusion coefficient and $M(C)$ is the rate of oxygen consumption per unit volume of cells as a function of the concentration C .

As the oxygen consumption rate of the oocyte is considerably different to that of the cumulus cells, the COC has been modelled as two spherically symmetric regions. The oxygen consumption rates in the cumulus and oocyte regions are taken to be $M_1(C_1)$ and $M_2(C_2)$ respectively where C_1 , C_2 are the O₂ concentrations in each of the regions. The cumulus mass is surrounded by follicular fluid that is assumed to have a known oxygen concentration C_f .

Thus, the oxygen concentration in the COC is found by solving two spherically symmetric diffusion equations, one for the cumulus mass yielding C_1 and the other for the oocyte yielding C_2 :

$$\frac{D}{r^2} \frac{d}{dr} \left(r^2 \frac{dC_1}{dr} \right) = M_1(C_1) \quad \text{for } a < r \leq b,$$

$$\frac{D}{r^2} \frac{d}{dr} \left(r^2 \frac{dC_2}{dr} \right) = M_2(C_2) \quad \text{for } 0 \leq r \leq a.$$

where:

- r is the radial distance from the centre of the oocyte,
- a is the radius of the oocyte,
- b is the radius of the COC,
- $C_1(r)$ is the concentration of oxygen in the cumulus mass,
- $C_2(r)$ is the concentration of oxygen in the oocyte,
- $M_1(C_1)$ is the rate of oxygen consumption per unit volume of cumulus mass,
- $M_2(C_2)$ is the rate of oxygen consumption per unit volume of oocyte.

Table 1 Morphological and oxygen consumption data for bovine and murine cumulus–oocyte complexes. The values for oxygen consumption within oocytes and cumulus cells were calculated from parameters within the table.

	Bovine	Murine
Radius of oocyte (m)		
Value	55 × 10 ^{−6}	40 × 10 ^{−6}
Reference	Gordon 2003	Chung 1973
Radius of COC (m)		
Value	160 × 10 ^{−6}	90 × 10 ^{−6}
Reference	Sutton-McDowall <i>et al.</i> 2004	Downs <i>et al.</i> 1997
Oocyte O ₂ uptake (pl/h)		
Value	240	140
Reference	Thompson <i>et al.</i> 1996	Houghton <i>et al.</i> 1996
COC O ₂ uptake (pl/h)		
Value	1873	3780
Reference	Sutton <i>et al.</i> 2003	Downs <i>et al.</i> 1997
Oocyte O ₂ consumption (mol/l of tissue/s)	3.8 × 10 ^{−6}	5.7 × 10 ^{−6}
Cumulus cell O ₂ consumption (mol/l of tissue/s)	1.1 × 10 ^{−6}	1.4 × 10 ^{−5}

At the boundary between the oocyte and the cumulus mass ($r = a$) we must have continuity of both mass flux and concentration, that is,

$$\frac{dC_1}{dr}(a) = \frac{dC_2}{dr}(a),$$

$$C_1(a) = C_2(a).$$

Also, the concentration must remain bounded at the centre of the COC (as $r \rightarrow 0$), or, equivalently for spherical symmetry,

$$\frac{dC_2}{dr}(0) = 0.$$

A further condition, on the boundary between the cumulus mass and the follicular fluid ($r = b$), governs the transport of oxygen into the COC. Here we assume that the flux of oxygen into the cumulus mass is proportional to the difference in oxygen concentration between the cumulus and the surrounding follicular fluid, that is,

$$-D \frac{dC_1}{dr}(b) = h(C_1(b) - C_f),$$

where h is the *transfer coefficient* for this problem.

A common approach to estimating oxygen concentration gradients in the biological literature (e.g. Gosden & Byatt-Smith 1986, Byatt-Smith *et al.* 1991) is to solve a diffusion-equation model with the rate of oxygen consumption assumed to be constant or proportional to the oxygen concentration. This assumption allows analytical solution of the governing equations. However, these solutions may be quite inaccurate, and even non-physical and hence invalid, in some regions of the parameter space, so that care must be exercised when using them. To overcome such difficulties, we have used a functional description of the consumption term that we believe more accurately represents the dependence of oxygen consumption rate on concentration, as we now describe.

In practice, cells consume oxygen at a maximal (or near to maximal) level, until oxygen concentration itself becomes rate limiting. The rate of consumption then falls with concentration, approaching zero as the O_2 concentration approaches zero. This behaviour is seen in the curves given in Jones (1984), which show oxygen concentration (O_2) versus cytochrome-c oxidation (as a measure of consumption rate) in hepatocytes.

A function that captures the general shape of these curves is the Arrhenius law from chemistry,

$$M(C) = \beta e^{-\alpha/C},$$

where α and β are positive constants. We use this function for the consumption terms in our equations, to relate the rate of oxygen consumption M to the

concentration C . As $C \rightarrow 0$, $e^{-\alpha/C} \rightarrow 0$ and, hence, $M(C) \rightarrow 0$, while as $C \rightarrow \infty$, $e^{-\alpha/C} \rightarrow 1$ and, hence $M(C) \rightarrow \beta$, i.e. β is the maximum rate of consumption. The constants α and β are chosen to fit appropriate experimental data. This function has the benefit of handling the changing oxygen consumption rate with concentration in a consistent manner for both the cumulus mass and oocyte, but also means that the equations cannot be solved analytically.

Our equations for oxygen transport in the COC are now

$$\frac{D}{r^2} \frac{d}{dr} \left(r^2 \frac{dC_1}{dr} \right) = \beta e^{-\alpha/C_1} \text{ for } a < r \leq b,$$

$$\frac{D}{r^2} \frac{d}{dr} \left(r^2 \frac{dC_2}{dr} \right) = \gamma e^{-\delta/C_2} \text{ for } 0 \leq r \leq a,$$

where α , β , γ and δ are positive constants. We have allowed that the constants in the consumption term may differ between the cumulus mass (α , β) and the oocyte (γ , δ). While not solvable analytically, this system of equations, together with the associated boundary conditions described earlier, is readily solved numerically using, for example, MATLAB (Version 6.5, 2002, The Mathworks Inc.).

Results

Estimation of model parameters from experimental data

Estimates of model parameters for cow and mouse were made using data from a variety of published sources (Table 1). In particular, using data on the size and total oxygen consumption by oocyte and COC, the maximum rate of oxygen consumption in moles (mol) per litre (l) of tissue per second (s) was computed thus: for a maximum oxygen uptake U picolitres per hour (pl/h) by a cell volume V cubic metres, the maximum oxygen consumption rate in mol per litre of tissue per second is given by

$$\frac{U \times 10^{-12}}{V \times 1000 \times 25.4 \times 3600}.$$

Here we have assumed oxygen to be an ideal gas so that the volume of a mole of oxygen at 37 °C is 25.4 litres. For the oocyte the uptake is as given in Table 1; for the cumulus it is the difference between that for the COC and that for the oocyte, i.e. $1873 - 240 = 1633$ pl/h. The volume of the oocyte is given by $V = 4\pi a^3/3$, where a is its radius in metres; the volume of the cumulus is $V = 4\pi(b^3 - a^3)/3$, where b is the radius of the COC in metres. The results of these calculations are shown in the bottom two rows of Table 1. It can be seen that a bovine oocyte consumes 3.5 times as much O_2 per unit volume of tissue per unit time than the surrounding cumulus cell

mass. In murine COCs this is reversed, with the cumulus cell mass consuming 2.4 times as much as the oocyte (per unit volume per unit time).

We set β to be the maximum rate of oxygen consumption by the cumulus mass (per unit volume per unit time), as given in Table 1 for each of cow and mouse. Since the consumption in a bovine (murine) oocyte is approximately 3.5 (0.4) times that in the cumulus (see Table 1), we take $\gamma = 3.5\beta$ and $\gamma = 0.4\beta$ for each of cow and mouse respectively.

The parameters α and δ in the consumption-rate terms determine the dependence of consumption rate on oxygen concentration, i.e. they are effectively measures of the half-maximal oxidation P_{50} value in the cumulus and oocyte respectively. We obtain estimates for these parameters by assuming, in the absence of specific data for the oocyte and cumulus, that they behave similarly to hepatocyte cells, and fitting the Arrhenius consumption-rate term to experimental data given in Jones (1984) which relates the rate of oxygen consumption to oxygen concentration for both untreated and digitonin-treated hepatocytes; digitonin-treated hepatocytes are shown to be less O_2 -dependent than untreated (intact) cells, with a P_{50} of about $2 \mu\text{mol L}^{-1}$ compared to $6 \mu\text{mol L}^{-1}$. The acellular oocyte can be expected to have similar O_2 dependence to digitonin-treated hepatocytes. Physiologically, the cumulus is likely to have a lower respiratory rate and, consequently, lower O_2 dependence than (untreated) hepatocytes; however, due to its cellular structure, the cumulus mass is likely to have greater O_2 dependence than digitonin-treated hepatocytes. Thus, the correct relation for the cumulus is expected to lie between these two curves, which may therefore be considered as limiting cases; intermediate cases may be obtained by varying the P_{50} value between that for digitonin-treated and that for untreated hepatocytes. In fact, the oxygen concentrations in the oocyte are lowest under the assumption that the cumulus has an O_2 dependence equal to digitonin-treated hepatocytes; as the P_{50} increases towards that for untreated hepatocytes, the concentration gradient across the COC decreases and oxygen concentrations in the oocyte increase because the cumulus mass consumes less oxygen. Therefore we have adopted the curve for digitonin-treated hepatocytes to represent the O_2 dependence of the cumulus as the 'worst-case' scenario in the sense that oxygen concentrations are not overestimated.

Thus, we use the curve for digitonin-treated hepatocytes in Jones (1984) to give the O_2 dependence for both the cumulus and oocyte (i.e. the values of α and δ in the consumption-rate terms of the model). With $P_{50} \approx 2 \mu\text{mol L}^{-1}$, the half-maximal consumption rate for the cumulus ($\beta/2$) is given by the Arrhenius law thus:

$$\frac{\beta}{2} = \beta e^{-\alpha/2 \times 10^{-6}}.$$

Dividing through by β and rearranging gives:

$$2 = e^{\alpha/2 \times 10^{-6}} \Rightarrow \frac{\alpha}{2} \times 10^6 = \ln(2)$$

$$\Rightarrow \alpha = 2\ln(2) \times 10^{-6} = 1.39 \times 10^{-6} \text{ mol L}^{-1}.$$

Thus we set $\alpha = \delta = 1.39 \times 10^{-6} \text{ mol L}^{-1}$ for both cow and mouse COCs.

As discussed later, because oxygen diffuses readily through cellular material and consumption terms for both the cumulus mass and the oocyte are specifically included in our equations, the appropriate diffusion coefficient is that of oxygen in physiological saline, i.e. $D = 2.5 \times 10^{-9} \text{ m}^2 \text{ s}^{-1}$, Jones (1984). The transfer coefficient h is a measure of the impedance to oxygen transport provided by the boundary between the follicular fluid and the cumulus mass; the higher the value, the lower the impedance. Because our model assumes the cumulus mass to be a homogeneous mixture of intra- and extracellular materials, there is no physical membrane boundary separating the cumulus mass from the follicular fluid and we expect little, if any, resistance to oxygen transport into the cumulus mass. Numerical experiments over a range of values of h revealed that there was little impedance for $h > 1$, effectively equivalent to setting the O_2 concentration at the boundary equal to the concentration in the follicular fluid (i.e. $C(b) = C_f$) and we have chosen to use a value of $h = 10 \text{ m/s}$. A summary of the parameter values used in our equations is given in Table 2.

The concentration of oxygen in the surrounding fluid (C_f) has a typical value (*in vivo*) of around 11% partial pressure (Knudsen *et al.* 1978, Fischer *et al.* 1992). However, our computations have been done for C_f in the range 3–20% in order to explore how the oxygen concentration in the follicular fluid affects concentrations in the COC. Concentrations in units of mole per litre (mol/l) for use in our model are found from 1% $O_2 = 7.6 \text{ mmHg}$ at sea level and the relationship:

Concentration of gas in a liquid (mol L^{-1})

$$= \text{Solubility (mol L}^{-1} \text{ mm Hg}^{-1}) \times \text{Pressure (mmHg)}$$

where solubility is taken to be $1.2 \times 10^{-6} \text{ mol/l}$ per mmHg.

Table 2 Parameters used for bovine and murine cumulus-oocyte complex.

Parameter	Bovine value	Murine value	Units
a	55×10^{-6}	40×10^{-6}	m
b	160×10^{-6}	90×10^{-6}	m
β	1.1×10^{-6}	1.43×10^{-6}	mol/l/s
γ	3.5β	0.4β	mol/l/s
α, δ	1.39×10^{-6}	1.39×10^{-6}	mol/l
D	2.5×10^{-9}	2.5×10^{-9}	m^2/s
h	10	10	M/s

Oxygen concentration: solutions

The governing equations were solved using MATLAB for each of two sets of parameters (for cow and mouse), and varying the oxygen concentration C_f in the surrounding fluid. Figure 1 shows the oxygen concentration gradient across a bovine COC when the oxygen concentration in the follicular fluid is at 10%. The shape of the curve is typical of other values also. Figure 2 shows the change in oxygen concentration with radius in the bovine cumulus mass only for a number of values of C_f , and Table 3 gives the oxygen concentration at the oocyte–cumulus boundary, for various values of C_f . As can be seen, there is a relatively small concentration gradient across the cumulus radius indicating that, in preovulatory antral follicles, a relatively small amount of the oxygen entering the COC is actually consumed by the cumulus mass, with the majority diffusing through to the oocyte.

Figure 3 and Table 3 give similar results for the murine COC. Even though the relative proportion of oxygen consumed per unit volume of cumulus mass is higher in a murine COC than a bovine, there is still very little (maximally approximately 0.5% O_2) consumed by the cumulus mass, because the cumulus volume is smaller than that of the oocyte.

Discussion

Diffusion models are appropriate for estimating concentration gradients in many cellular systems. Early one-dimensional steady-state models are found in Krogh (1919) and Warburg (1923). Krogh (1919) considered oxygen diffusion from a cylindrical capillary into the surrounding tissue; oxygen concentration was assumed to be independent of distance along the axis of the

Figure 1 O_2 concentration C/C_f versus radius r/b in the bovine cumulus–oocyte complex for an oxygen concentration $C_f=10\%$ in the surrounding follicular fluid. (A) Oocyte region, (B) cumulus region. The dotted line denotes the boundary between the cumulus mass and the oocyte.

Figure 2 O_2 concentration C_1/C_f versus radius r/b in the bovine cumulus mass for various concentrations of oxygen in the surrounding follicular fluid: (A) $C_f=20\%$, (B) $C_f=10\%$ and (C) $C_f=5\%$.

capillary and angular position and, hence, to vary only with radial distance from the capillary wall. Warburg’s (1923) model assumes that concentration is constant in a plane and varies in the direction normal to the plane. More recently, the spherically symmetric form of the steady-state diffusion equation has been employed for modelling oxygen concentrations in ovarian follicles (Gosden & Byatt-Smith 1986) and in preimplantation embryos in static culture (Byatt-Smith *et al.* 1991).

Our model also assumes spherical symmetry, but differs from previous work in that we have incorporated a term that allows for nonlinear variation of the oxygen consumption rate with oxygen concentration in a manner that fits experimental data. Previous studies (Gosden & Byatt-Smith 1986, Byatt-Smith *et al.* 1991) consider the cases of constant rate of oxygen consumption and consumption rate proportional to concentration, which fail to give accurate solutions at low and high oxygen concentrations respectively. Gielen & Kranenbarg (2002) combined these two cases when

Table 3 The concentration of O_2 at the surface of a bovine and murine oocyte for various values of O_2 concentration in the surrounding follicular fluid.

% O_2 in follicular fluid C_f	Proportion at bovine oocyte $C_1(a)/C_f$	% O_2 at bovine oocyte–cumulus boundary $r=a$	Proportion at murine oocyte $C_1(a)/C_f$	% O_2 at murine oocyte–cumulus boundary $r=a$
20	0.987	19.74	0.972	19.44
10	0.975	9.75	0.944	9.44
7	0.964	6.75	0.921	6.45
6	0.958	5.75	0.908	5.45
5	0.950	4.75	0.890	4.45
4	0.938	3.75	0.864	3.46
3	0.919	2.76	0.822	2.47

Figure 3 O_2 concentration C_1/C_f versus radius r/b in the murine cumulus mass for various concentrations of oxygen in the surrounding follicular fluid: (A) $C_f=20\%$, (B) $C_f=10\%$ and (C) $C_f=5\%$.

considering oxygen diffusion in small organisms; O_2 consumption was taken to be piecewise linear-constant at high O_2 concentrations, and proportional to concentration below some threshold O_2 concentration. This is more accurate, but requires that the boundary between regions of high and low O_2 concentration be determined (e.g. by solving a complex transcendental equation). By introducing a nonlinear term to represent the rate of oxygen consumption as a function of O_2 concentration, we eliminate the need to determine regions of high and low concentration and have constructed a model that can be better fitted to experimental data and hence should be more accurate over the entire range of possible oxygen concentrations.

Our model also differs from these previous similar studies in considering two regions (oocyte and cumulus) sharing a common boundary, both of which consume oxygen at different nonlinear rates. Multi-compartment models can be found in the literature to represent nutrient diffusion in other biological systems (for example Hoofd *et al.* 1990, Bruggeman *et al.* 2001, Kumar *et al.* 2004) but have not been applied to cumulus–oocyte complex systems.

Our results for both cow and mouse show a small concentration gradient from the follicular fluid across the cumulus mass to the oocyte, and hence demonstrate that only a small fraction of the oxygen entering the COC system is consumed by the cumulus mass during diffusion across the cumulus layer. Thus the level of oxygen at the surface of the oocyte is approximately 5–15% lower than that in the follicular fluid, depending on O_2 concentration and metabolic activity of cumulus cells between species. However, this result is sensitive to the value of the diffusion coefficient D for oxygen across the oocyte–cumulus complex.

There has been much discussion in the literature on the appropriate value of the oxygen diffusion coefficient

for a medium consisting largely of intracellular matter/fluid (Hills 1970, Tai & Chang 1974, Jones & Kennedy 1982, Jones 1986, McCabe *et al.* 2003). Values ranging from that for pure water at 37 °C ($D_w = 2.5 \times 10^{-9} \text{ m}^2/\text{s}$) to 3 orders of magnitude smaller have been used. For example, Gosden & Byatt-Smith (1986) used a value of $7 \times 10^{-12} \text{ m}^2/\text{s}$, but later (Byatt-Smith *et al.* 1991) considered D_w as well as values 5–10 times smaller. Our review of the literature leads us to conclude that values of the diffusion coefficient differing significantly from D_w are due to assumptions made in deriving the models used to determine the diffusion coefficient from experimental data, as well as error in experimental measurement. For example, the common neglect of oxygen consumption by some parts of a biological system will result in a low apparent diffusion coefficient. We note that Jones (1986), commenting on a diffusion coefficient $\sim D_w/70$, states that that “no physical or chemical basis is known to account for such a low value in cells”. Jones (1986) also quotes experimental studies that indicate a value $\sim 10^{-9} \text{ m}^2 \text{ s}^{-1}$ for the diffusion coefficient of oxygen in tissue, which corresponds to D_w . Recently, McCabe *et al.* (2003) discussed the experimental evidence for enhanced oxygen diffusion in respiring tissue, such as in a COC, and quote a diffusivity of $D = 3 \times 10^{-9} \text{ m}^2 \text{ s}^{-1}$ for liver cells. This is not significantly different (only 1.2 times) to the value in water used here. An even more recent study of oxygen levels in a very different system, the mammalian ocular lens containing closely packed fibre cells (McNulty *et al.* 2004), finds a diffusion coefficient “indistinguishable from that of oxygen in water” using a spherically symmetric diffusion model including consumption. Since our model includes specific terms for all oxygen consumption by both cumulus mass and oocyte, we conclude that the value of the diffusion coefficient appropriate to our model is that for oxygen diffusion in pure water at 37 °C, which is also the value quoted for oxygen diffusion in physiological saline.

The significance of our results should have impact in several areas of oocyte biology and maturation. First, as already mentioned, in studying O_2 diffusion across a pre-antral follicle, Gosden & Byatt-Smith (1986), with reference to Jones and Kennedy (1982), used a diffusion coefficient of $7 \times 10^{-12} \text{ m}^2/\text{s}$, almost 3 orders of magnitude smaller than that of O_2 in water. This results in a considerably steeper concentration gradient across the oocyte–granulosa cell complex, and hence a much lower oxygen concentration at the oocyte–granulosa cell boundary, than is obtained using the diffusion coefficient for O_2 in water (data not shown). The small diffusion coefficient was, in fact, obtained by comparing experimental data for rat hepatocytes with predictions from a model that assumes no oxygen consumption in a rat hepatocyte excepting for that by an isolated mitochondrion at the centre of the cell (see Jones & Kennedy, 1982). This will result in a significant underestimation of

the diffusion coefficient and, consequently, low predictions of oxygen concentrations from models that use it. Hence, the results from Gosden & Byatt-Smith (1986), which suggest that the developing oocyte in the pre-antral follicle is certainly anoxic, should be reviewed.

Secondly, there is no consensus as to the optimal O_2 concentration for *in vitro* maturation of oocytes. The early embryo appears to be sensitive to either atmospheric or hypoxic O_2 levels in several mammalian species, resulting in decreased development (e.g. Thompson *et al.* 1990). Intermediate concentrations of O_2 , which appear to approximate *in vivo* concentrations, are beneficial for development (Harvey *et al.* 2002). Therefore, it seems likely that O_2 levels for *in vitro* maturation should follow a similar trend, in that levels approximating *in vitro* concentrations would be more likely to be beneficial for oocyte maturation, rather than the majority current practise of the use of atmospheric levels. Recently, Hashimoto *et al.* (2000) demonstrated that for *in vitro* maturation of bovine oocytes in the presence of 20mM glucose, 5% O_2 during maturation yielded more blastocyst-stage embryos than 20% O_2 . This also correlated with a reduction in the intra-oocyte level of reactive oxygen species. The relatively high glucose level was required to overcome deficiencies in meiotic maturation, which are glucose concentration-dependent during *in vitro* maturation (Sutton-McDowell *et al.* 2005). Such changes in substrate concentration may alter oxygen consumption rates by the COC. However, we believe that such changes would lead to only small deviations in oxygen uptake, as many more molecules of substrates such as pyruvate and glucose are oxidised for every additional molecule of oxygen consumed.

One critical assumption made for our mathematical model is that the COC is close to spherical. This is clearly not the case, where a "stalk" of granulosa cells fixes the cumulus mass to the membrana granulosa. Around this region, one would expect the O_2 concentration at the surface of the oocyte to be less than indicated by the values given in this paper, allowing the establishment of a polarised O_2 concentration around the oocyte. Whether or not such a gradient has physiological relevance is unknown, although the concept of localised intracellular ATP production and polarity in mitochondrial distribution is clearly discussed in the literature (e.g. for review, see Van Blerkom 2004).

In conclusion, we have developed a mathematical model to describe the perfusion of oxygen across the COC. This model reveals that follicle lumen concentrations of oxygen most likely reflect what is seen by the oocyte itself. Although it is known that the cumulus-free oocyte is dependent on oxidative phosphorylation for developmental competency (Van Blerkom *et al.* 1995), dependency of the oocyte on its own mitochondrial activity during maturation, especially when significant cell-communication occurs via gap

junctions with the cumulus layer, is unknown (Van Blerkom 2004). However, even if oxygen consumption by the oocyte is less than the values utilised here, the gradient of O_2 would be even less than our current model predicts. Overall, we believe our model makes physiological sense and therefore may be useful in determining the sensitivity of maturing COCs to different oxygen concentrations. Furthermore, our model can also be applied to other questions relating to diffusion of molecules across spherical structures occurring within reproduction, such as follicles and early embryos.

Acknowledgements

ARC is supported by a University of Adelaide scholarship (Adelaide Scholarships International, ASI). This work was partially supported by NIH as part of the NICHD National Cooperative Program on Female Health and Egg Quality under cooperative agreement U01 HD044644. The authors are thankful for some helpful comments from Dr S. Cox (Applied Mathematics, The University of Adelaide).

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Received 20 September 2005

First decision 17 November 2005

Revised manuscript received 14 December 2005

Accepted 21 February 2006