Oxygen and the ovarian follicle

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Gabe Peter Redding

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Abstract

The role oxygen plays in the developing ovarian follicle is of interest not only to the field of developmental biology but also to in-vitro fertilisation (IVF) technologists, as oxygenation of the oocyte is considered to be a potential determinant of oocyte competence.

Oxygen transport through the developing ovarian follicle, and practical aspects of the analysis of oxygen in human follicular fluid were investigated in this work.

Mathematical modelling of oxygen transport in the pre-antral, and antral/pre-ovulatory follicle revealed a number of interesting findings.

Contrary to previous conclusions (Gosden & Byatt-Smith, 1986), oxygen can reach the oocyte in the small pre-antral follicle. Improved estimates of diffusion coefficients through the granulosa cell layer and the inclusion of fluid voidage in this layer showed that oxygen can also reach the oocyte in large pre-antral follicles. The amount of oxygen that reaches the oocyte in the pre-antral follicle is a function of its size and degree of vascularisation. Symmetrically distributed vascularisation is superior in achieving a well oxygenated follicle.

However, the large pre-antral follicle will eventually reach a size beyond which it cannot grow without anoxic regions developing. The size at which this occurs is consistent with the size at which antrum formation is observed in human follicles.

The model predicts that the follicle can avoid an anoxic state through antrum formation, and shows that the follicle develops in a way that is consistent with overcoming mass transport limitations. The oxygen status of the follicle during the antral/pre-ovulatory phase of growth requires that the volume of granulosa cells be balanced by the volume of follicular fluid.

Further predictions suggest that oocyte respiration becomes sub-maximal at follicular fluid volumes below approximately 4ml, vascularisation levels below 38%, or fluid

dissolved oxygen levels below 5.1 vol%. These values are consistent with observations in the literature. It was also shown that the measurement of follicular fluid dissolved oxygen levels could provide a simple measure of the respiratory status of the oocyte, and this may be superior to the measurement of follicular vascularisation which requires knowledge of more parameters.

Methodology for the analysis of follicular fluid oxygen solubility and diffusivity was developed using a Clark oxygen electrode. Analysis of these parameters showed that they are similar to human plasma, and allowed the predictive uncertainty of the model to be reduced.

Experimental studies into the effects of IVF aspiration on follicular fluid were carried out. Aspiration results in significant changes in the properties of follicular fluid. Dissolved oxygen levels rose 5 ± 2 vol%, pH increased by 0.04 ± 0.01 pH units, and temperature dropped by 7.7 ± 1.3 °C. Mathematical modelling of blood contaminated follicular fluid also showed that contamination results in significant changes in the dissolved oxygen of the fluid. This suggests that if the composition of follicular fluid is to be determined (particularly dissolved oxygen), sampling and/or measurement of fluid must take place before the collection vial of the aspiration kit, and blood contamination must be eliminated.

Based on this result, the design and testing of devices capable of reliable sampling and/or measurement of oxygen levels of follicular fluid was considered. This presents a continuing challenge, including the integration of routine follicular fluid oxygen measurement into clinical practice.

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I feel obligated by tradition to say something profound in the final paragraph of the acknowledgements section. Unfortunately I don't seem to have any runners on base in that department today. I am all spent for the next little while. So, a joke instead (somebody else's), which is best read aloud,

Why did the sperm cross the cumulus?

To get to the oocyte.

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NOMENCLATURE

Note that some symbols which are used only once are defined in the text where they occur and are not included on this list.

A	cathode surface area	m^2
Bi	dimensionless ratio of membrane to sample layer resistance to mass transport	
c_p	heat capacity	$J.kg^{-1}.K^{-1}$
c_{pff}	heat capacity of follicular fluid	$J.kg^{-1}.K^{-1}$
C	oxygen concentration	$mol.m^{-3}$
C_o	oxygen concentration at the follicle surface	$mol.m^{-3}$
C_{crit}	Oxygen concentration of critical interest (most notably the critical oxygen concentration at which oocyte is only just respiring maximally ($R_o = 0.99R_{omax}$))	$mol.m^{-3}$
C_{0mean}	the mean surface concentration of a partially vascularised follicle	$mol.m^{-3}$
$C_{arterial}$	oxygen concentration in the plasma portion of arterial blood	$mol.m^{-3}$
C_{novasc}	oxygen concentration at a un-vascularised surface of the follicle	$mol.m^{-3}$
C_i	oxygen concentration at the granulosa/antrum interface	$mol.m^{-3}$
C_{imin}	minimum oxygen concentration required at the granulosa/antrum interface to sustain the oocyte at R_o=0.99R_{omax}	$mol.m^{-3}$
C_{omin}	minimum oxygen concentration required at the follicle surface to sustain the oocyte at R_o=0.99R_{omax}	$mol.m^{-3}$
C_{ii}	concentration of oxygen at the cumulus/antrum interface	$mol.m^{-3}$
C_a	analyte concentration	$mol.m^{-3}$
C_p	analyte concentration in plasma	$mol.m^{-3}$
C_f	analyte concentration in follicular fluid	$mol.m^{-3}$

C_H	concentration of haemoglobin in blood	$mol.m^{-3}$
d_{min}	minimum distance from oocyte centre to outer edge of either compact or expanded cumulus mass	mm
d_{max}	maximum distance from oocyte centre to outer edge of either compact or expanded cumulus mass	mm
d_f	follicle diameter	m
D_g	diffusion coefficient of oxygen in the granulosa cell layer	$m^2.s^{-1}$
D_{cell}	diffusion coefficient of oxygen through the cellular fraction of tissue	$m^2.s^{-1}$
D_p	diffusion coefficient of oxygen in plasma	$m^2.s^{-1}$
D_{eff}	effective diffusion coefficient of oxygen through tissue	$m^2.s^{-1}$
D_a	diffusion coefficient of oxygen in the antral fluid	m
D_c	diffusion coefficient of oxygen in the cumulus cell layer	$m^2.s^{-1}$
D_m	diffusivity of oxygen in the membrane of a dissolved oxygen electrode	$m^2.s^{-1}$
D_s	diffusivity of oxygen in the sample solution	$m^2.s^{-1}$
D_w	diffusivity of oxygen in water	$m^2.s^{-1}$
D_{rel}	relative diffusivity of oxygen in sample compared to water	
F	Faradays constant (number of Coulombs per mol of electrons)	$coulombs.mol^{-1}$
ΔH_{vap}	enthalpy of vaporisation of water	$J.mol^{-1}$
I	current produced by Clark oxygen electrode	A
I_g	steady-state gas phase current	A
I_s	steady-state current with sample solution layer in place	A
I_w	steady-state current with water layer in place	A
js	number of nodes in the sample solution layer	
jm	number of nodes in the membrane layer	
J	oxygen flux	$mol.s^{-1}$
K_1	first integration constant	m^{-2}
K_2	second integration constant	$mol.m^{-3}$
K_m	Michaelis-Menton constant	$mol.m^{-3}$

L_m	membrane thickness	m
L_s	sample thickness	m
L_w	water layer thickness	m
m	flow rate	$kg.s^{-1}$
n	number of electrons involved in the reduction of oxygen at the cathode	e
M_{O2}	molecular mass of oxygen	$g.mol^{-1}$
p	oxygen partial pressure	mmHg
p_o	oxygen partial pressure at follicle surface	mmHg
p_i	partial pressure of oxygen at the granulosa/antrum interface or membrane/sample interface	mmHg
p_g	partial pressure of oxygen in the gas phase	mmHg
p_m	partial pressure of oxygen in the membrane	mmHg
p_s	partial pressure of oxygen in the sample solution	mmHg
pO_2	partial pressure of oxygen	mmHg
pO_{2b}	partial pressure of oxygen in blood	mmHg
pO_{2f}	partial pressure of oxygen in follicular fluid	mmHg
pO_{2m}	partial pressure of oxygen in blood/follicular fluid mixture	mmHg
P_1	number of pixels occupied by oocyte	pixels
P_2	number of pixels occupied by oocyte and compact cumulus	pixels
P_3	number of pixels occupied by cumulus-oocyte complex (cells and fluid)	pixels
P_4	number of pixels occupied by cumulus-oocyte complex (cells only)	pixels
P_{ATM}	atmospheric pressure	atm
P_m	permeability of oxygen in the membrane of a dissolved oxygen electrode	$mol.m^{-1}.s^{-1}.mmHg^{-1}$
P_s	permeability of oxygen in the sample solution	$mol.m^{-1}.s^{-1}.mmHg^{-1}$
P_w	permeability of oxygen in water	$mol.m^{-1}.s^{-1}.mmHg^{-1}$
P_{rel}	relative permeability of oxygen in sample compared to water	

P_{50}	partial pressure of oxygen at which 50% of haemoglobin oxygen binding sites are full	mmHg
r	radial distance from follicle centre	m
r_f	follicle radius	m
r_o	oocyte radius (note this parameter expressed in mm in Chapters 7 and 8)	m
r_{anoxic}	distance from follicle centre at which follicle becomes depleted of oxygen	m
r_{fcrit}	critical follicle radius beyond which no oxygen will reach the surface of the oocyte.	m
r_a	distance from the follicle centre to the granulosa/antrum interface	m
r_c	distance from the follicle centre to the cumulus/antrum interface	m
R_{gmax}	maximal oxygen consumption rate by the granulosa cells	$mol.m^{-3}.s^{-1}$
R_g	oxygen consumption rate of the granulosa cells	$mol.m^{-3}.s^{-1}$
R_{omax}	maximal oxygen consumption rate by the oocyte	$mol.m^{-3}.s^{-1}$
R_o	oxygen consumption rate by the oocyte	$mol.m^{-3}.s^{-1}$
R	gas constant	$L.atm.mol^{-1}.K^{-1}$
R_a	oxygen consumption rate in the fluid antrum	$mol.m^{-3}.s^{-1}$
R_c	oxygen consumption rate of the cumulus cells	$mol.m^{-3}.s^{-1}$
S	solubility of oxygen in solution	$mol.m^{-3}.mmHg^{-1}$
S_a	solubility of oxygen in the antral fluid	$mol.m^{-3}.mmHg^{-1}$
S_m	solubility of oxygen in the membrane of a dissolved oxygen electrode	$mol.m^{-3}.mmHg^{-1}$
S_s	solubility of oxygen in the sample solution	$mol.m^{-3}.mmHg^{-1}$
S_w	solubility of oxygen in water	$mol.m^{-3}.mmHg^{-1}$
S_{rel}	relative solubility of oxygen in sample compared to water	
S_p	solubility of oxygen in plasma	$mol.m^{-3}.mmHg^{-1}$
t	time	s
t_f	time required for follicular fluid sample to travel through viscometer	s
t_w	time required for water to travel through viscometer	s

T	temperature	K
U_{min}	minimum gas stream velocity required to produce sustained waves on a flat liquid surface	$cm.s^{-1}$
$\%vasc$	mean symmetrical vascularisation	%
V_g	volume of granulosa cells in follicle	m^3
V_{cell}	volume of a single granulosa cell	$m^3.cell^{-1}$
V_f	total volume of follicle	m^3
V_a	volume of antral fluid	m^3
V_o	volume of the oocyte	m^3
V_b	volume of blood contamination	m^3
V_p	volume of plasma	m^3
V_{ff}	volume of follicular fluid	m^3
V_{RBC}	total volume of red blood cell sediment	m^3
x	position in membrane/sample solution system	m
x'	position in sample layer of analytical solution	m
X	weight fraction	
Y_H	fraction of haemoglobin saturated by oxygen	
y	mass of evaporated fluid	kg
Y	mass of aspirated fluid	kg
Φ	cell fraction	
Φ_L	heat flux	W
ρ_f	density of follicular fluid	$kg.m^{-3}$
ρ_w	density of water	$kg.m^{-3}$
ρ_{O2}	density of oxygen gas	$g.L^{-1}$
ε	fluid voidage	
τ	dimensionless time	
μ_a	kinematic viscosity of follicular fluid	mm^2s^{-1} (cSt)
μ_w	kinematic viscosity of water	mm^2s^{-1} (cSt)
θ	temperature	°C
$\Delta\theta$	temperature change	°C
$\Delta\theta_{tot}$	total temperature change over entire aspiration kit	°C
$\Delta\theta_1$	temperature drop over the collection vial when aspiration is performed normally	°C

$\Delta\theta_2$	temperature drop over the collection vial when the collection vial is heated and then insulated prior to aspiration	°C
$\Delta\theta_3$	temperature drop over the collection vial when the collection vial is insulated only prior to aspiration	°C