

Copyright is owned by the Author of the thesis. Permission is given for a copy to be downloaded by an individual for the purpose of research and private study only. The thesis may not be reproduced elsewhere without the permission of the Author.

Oxygen and the ovarian follicle

A thesis presented in partial fulfilment of the requirements for the degree of
Doctor of Philosophy
in
Bioprocess Engineering

At Massey University, Palmerston North,
New Zealand

Gabe Peter Redding

2007

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.

Acknowledgements

The work reported here was done in partial fulfilment of the contract: C10X0204, 'Advanced Tools for the Problem of Infertility in Women', awarded to AgResearch Ltd. by the New Zealand Foundation for Research Science and Technology.

I would like to thank all the staff and students at Massey University and AgResearch who have helped me during this project. Your number is too many to thank personally.

Many thanks to my supervisors, Dr. John Bronlund, and Dr. Alan Hart. John, thank you for the academic assistance, especially for focusing me on what matters. Alan, although your assistance was often academic I am particularly grateful for the day to day practical assistance and encouragement you provided me, which enabled me to get the job done. As supervisors your skills are very complimentary. To Wendy Collier thank you for all your help, and for putting up with me in general. It must surely be my turn to do the dishes now.

To all the staff at the ISIS clinic in Hamilton and in the Dairy Science building at Ruakura, thanks for having me, and making me feel welcome.

My family, Mum, Dad, Cain, Janell, and Fizz. Thank you for your support, but also for not asking too often how the thesis was going. Without you achievement is worth very little. Janell, thank you for your love and support. Without you, I simply would not have been able to finish this work.

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.

TABLE OF CONTENTS

Chapter 1	Introduction and objectives	1
Chapter 2	Review of the literature	4
2.1	Human reproduction	4
2.1.1	The female reproductive tract	4
2.1.2	Follicle development	5
2.2	The in-vitro fertilisation process	9
2.2.1	The steps of the IVF procedure	13
2.2.1.1	Use of drugs to stimulate ovaries	13
2.2.1.2	Collection of eggs from the ovaries	14
2.2.1.3	Fertilisation and embryo development	16
2.2.1.4	Transfer of embryos to the uterus	18
2.2.1.5	Use of drugs to increase receptiveness of uterus to embryos	19
2.3	Follicular fluid	19
2.3.1	Physical properties and composition	21
2.3.2	pH and oxygen partial pressure (pO_2)	21
2.3.3	Electrolytes	23
2.3.4	Glucose and organic acids	23
2.3.5	Proteins	24
2.4	Prediction of oocyte quality	24
2.4.1	Oxygen concentration in follicular fluid as a predictor of oocyte quality	27
2.4.2	Perifollicular blood flow and oocyte quality	27
2.4.3	Other predictive measures	28
2.5	Issues specific to the analysis of dissolved oxygen in follicular fluid	30
2.5.1	Blood contamination	30
2.5.2	Fouling	30
2.5.3	Anaesthetic interference	31
2.5.4	Oxygen uptake post aspiration	32
2.5.5	Oocyte shear	32

2.5.6	Sterilization	33
2.6	Conclusions and recommendations	33
Chapter 3	Modelling oxygen transport in the pre-antral follicle	36
3.1	The pre-antral follicle	36
3.2	The model of Gosden & Byatt-Smith (1986)	36
3.3	Model improvement	44
3.3.1	Parameter estimation and variation	44
3.3.1.1	Estimation of the oxygen concentration at the follicle surface (C_o)	47
3.3.1.2	Estimation of rate of oxygen consumption by granulosa cells (R_g)	47
3.3.1.3	Estimation of follicle radius (r_f)	48
3.3.1.4	Estimation of oxygen diffusivity in the granulosa cell layer (D_g)	48
3.3.1.4.1	Theoretical analysis	48
3.3.1.4.2	Experimental analysis	50
3.3.2	Sensitivity analysis	53
3.3.3	Assumption of no fluid voidage	65
3.3.4	The assumption of no oocyte	69
3.3.5	The assumption of constant oxygen consumption rate of the granulosa cells (R_g)	72
3.3.6	The assumption of complete vascularisation	78
3.3.7	Variability in vascular distribution	80
3.4	Conclusions and recommendations	94
Chapter 4.	Modelling oxygen transport in the antral / pre-ovulatory follicle	98
4.1	The antral and pre-ovulatory follicle	98
4.2	Antral follicle model – description and assumptions	99
4.3	Antral follicle model derivation	100
4.3.1	Estimation of the volume of granulosa cells	109
4.3.2	Oxygen supply to the oocyte and Michaelis-Menton kinetics	116

4.3.2.1 Michaelis-Menton kinetics and the antral/pre-ovulatory follicle	121
4.3.3 Effect of reduced vascularisation	125
4.3.3.1 Effect of variable vascular distribution on oxygen concentration profiles in the antral fluid	133
4.4 Pre-ovulatory follicle model – description and assumptions	133
4.5 Pre-Ovulatory follicle model derivation	134
4.5.1 Antral fluid	134
4.5.2 In the granulosa cell layer	139
4.5.3 Vascularisation and required interfacial oxygen concentration	150
4.5.4 Effect of addition of cumulus cells	157
4.5.4.1 Model derivation	158
4.5.4.1.1 Cumulus Layer	158
4.5.4.1.2 Antral Fluid	161
4.5.4.1.3 Granulosa cell layer	163
4.5.4.2 Estimation of r_c , R_c , and D_c	165
4.5.5 Effect of variability in follicular fluid solubility	170
4.6 Conclusions and recommendations	175
 Chapter 5. Methodology for the analysis of the permeability, diffusivity and solubility of oxygen in aqueous fluids	 177
5.1 Methods for the measurement of gas diffusivity in liquids	177
5.2 Materials and methods	179
5.3 Steady-state analysis	182
5.4 Transient analysis	188
5.4.1 Transient model development	188
5.4.2 Switch on	189
5.4.3 Steady-state sample addition	192
5.4.4 Up-step	193
5.4.5 Down-step	194
5.4.6 Model solution	195
5.4.7 Comparison of techniques	200
5.5 Analytical solution development	203

5.5.1	Down-step experiment analytical solution	208
5.5.2	Sample steady-state addition experiment analytical solution	209
5.6	Data analysis	210
5.7	Validation of methodology against standard solutions	211
5.8	Conclusions and recommendations	222
Chapter 6.	The physical and transport properties of human follicular fluid	223
6.1	Materials and methods	223
6.2	Results and discussion	226
6.2.1	Density, osmolality, and viscosity	226
6.2.2	Oxygen permeability, diffusivity, and solubility	231
6.2.2.1	The effect of variability in follicular fluid oxygen diffusivity and solubility on the measurement of follicular fluid dissolved oxygen	233
6.3	Conclusions and recommendations	238
Chapter 7.	Follicular fluid changes during IVF aspiration	239
7.1	Materials and methods	240
7.1.1	Aspiration system	240
7.1.2	Follicular fluid	240
7.1.3	Oxygen measurements	242
7.1.4	pH measurements	242
7.1.5	Temperature measurements	243
7.1.6	Cell shear measurements	243
7.2	Results and discussion	249
7.2.1	Dissolved oxygen changes during aspiration	249
7.2.2	pH changes during aspiration	253
7.2.3	IVF aspiration and blood contamination	253
7.2.3.1	Blood contamination and follicular fluid dissolved oxygen	255
7.2.3.2	Blood contamination and other analytes	261
7.2.4	Temperature changes during aspiration	262
7.2.5	Effects of IVF aspiration on the Cumulus-oocyte complex	273
7.3	Conclusions and recommendations	282

Chapter 8.	Practical aspects of the sampling and analysis of dissolved oxygen in follicular fluid	284
8.1	Materials and methods	284
8.2	Follicular fluid sampling alternatives	285
8.2.1	System constraints	287
8.2.2	In-line sampling device design and testing	292
	8.2.2.1 Sampling with unchanged oxygen levels	296
	8.2.2.2 Sampling without shear damage to the oocyte	298
8.2.3	From prototype to clinic	303
8.2.4	In-line measurement	309
8.3	Conclusions and recommendations	311
Chapter 9.	Conclusions and recommendations	312
Chapter 10.	References	315
Appendix A.	Procedure for the maturation of bovine cumulus-oocyte complexes	334

LIST OF FIGURES

Figure 2.1	Structure of the female reproductive tract (taken from Findlay 1984) and the ovary (taken from Sloane 1985).	6
Figure 2.2	Relationship between the pituitary and the ovaries, indicating the circulation of hormones between the pituitary gland and the ovaries (taken from Stangel, 1979).	10
Figure 2.3	Follicle growth and development (taken from Shostak, 1991).	11
Figure 2.4	IVF aspiration for oocyte collection using ultrasound guidance (supplied by Fertility Associates Inc.).	17
Figure 2.5	Typical IVF aspiration kits for both single (a) and double lumen needles (b) (SwedMed International).	20
Figure 2.6	Comparison of reported values of dissolved oxygen levels in follicular fluid.	25
Figure 3.1	Development of the pre-antral follicle in humans.	37
Figure 3.2	Pictorial representation of the model of oxygen transport in the pre-antral follicle proposed by Gosden & Byatt-Smith (1986). Symbols defined in nomenclature. The oocyte is assumed to have the same properties as the granulosa cells and therefore its dimensions are not included.	38
Figure 3.3	Diffusion through an internal shell (shaded) of a spherical follicle.	41
Figure 3.4	Oxygen concentration as a function of distance from the centre of a pre-antral follicle at the parameter values given by Table 3.1.	46
Figure 3.5	Comparison of measured values of D_{cell} from the literature with the range predicted in this work.	55
Figure 3.6	The effect of variation of C_0 on the distance from the follicle surface at which follicle becomes anoxic (note that r/r_f has a value of 1 at the follicle surface).	57
Figure 3.7	The effect of variation of R_g on the distance from the follicle surface at which follicle becomes anoxic (note that r/r_f has a value of 1 at the follicle surface).	58

Figure 3.8	The effect of variation of r_f on the distance from the follicle surface at which follicle becomes anoxic (note that r/r_f has a value of 1 at the follicle surface).	59
Figure 3.9	The effect of variation of D_g on the distance from the follicle surface at which follicle becomes anoxic (note that r/r_f has a value of 1 at the follicle surface).	60
Figure 3.10	The effect of variation of D_g over the range tested by Gosden & Byatt-Smith (1986) on the distance at which the follicle becomes anoxic (note that r/r_f has a value of 1 at the follicle surface).	61
Figure 3.11	Oxygen concentration profiles across a small ($r_f=25 \mu\text{m}$) pre-antral follicle using parameter values favourable and unfavourable to oxygen transport.	63
Figure 3.12	Oxygen concentration profiles across a large ($r_f=200 \mu\text{m}$) pre-antral follicle using parameter values favourable and unfavourable to oxygen transport.	64
Figure 3.13	The effect of fluid voidage on the effective diffusion coefficient (D_{eff}) at low ($0.1 \times 10^{-5} \text{ cm}^2 \cdot \text{s}^{-1}$) and high ($0.5 \times 10^{-5} \text{ cm}^2 \cdot \text{s}^{-1}$) values of D_{cell} according to equation 3.20. Included is the value for the oxygen diffusion coefficient in human plasma (D_p).	67
Figure 3.14	Effect of fluid voidage on the distance from the follicle centre at which a large ($r_f=200 \mu\text{m}$) pre-antral follicle becomes anoxic using parameter estimates favourable and unfavourable to oxygen transport.	70
Figure 3.15	Critical follicle radius beyond which no oxygen reaches oocyte surface, as a function of fluid voidage using parameter estimates favourable and unfavourable to oxygen transport ($r_o=40 \mu\text{m}$).	71
Figure 3.16	Oxygen concentration profiles in a large pre-antral follicle under the assumption of constant granulosa cell oxygen consumption (R_g) compared with Michaelis-Menton kinetics.	76
Figure 3.17	Concentration dependence of oxygen consumption described by Michaelis-Menton kinetics (not to scale).	77

- Figure 3.18 Relationship between mean symmetrical % vascularisation and the distance at which a small ($r_f=25 \mu\text{m}$) pre-antral follicle becomes anoxic for favourable and unfavourable parameter estimates. Note that using favourable estimates less vascularisation is required to maintain oxygen at the oocyte surface ($\sim 3\%$ compared to $\sim 30\%$). 82
- Figure 3.19 Critical follicle radius beyond which no oxygen reaches oocyte surface as a function of fluid voidage using parameter estimates favourable to oxygen transport ($r_o=40 \mu\text{m}$) at various vascularisation levels. Intersections of grey lines illustrate two example follicles each with a radius of $200 \mu\text{m}$ and with differing voidage of 0.3 and 0.7. 83
- Figure 3.20 Critical follicle radius beyond which no oxygen reaches oocyte surface as a function of fluid voidage using parameter estimates unfavourable to oxygen transport ($r_o=40 \mu\text{m}$) at various vascularisation levels. Intersection of grey line illustrates an example follicle with a radius of $200 \mu\text{m}$ and a voidage of 0.6. 84
- Figure 3.21 Critical follicle radius beyond which the oocyte will receive no oxygen ($r_o = 40 \mu\text{m}$) at vascularisation increments consistent with Femlab's spherical divisions and at nominal values for oxygen transport. Illustrated is the selection of a follicle with 0.3 voidage and 50% vascularisation. 86
- Figure 3.22 Oxygen concentration in a $108 \mu\text{m}$ radius pre-antral follicle with various vascular distributions (25% vasc., $\varepsilon = 0.3$). Concentration scales have units of mol.m^{-3} . 89
- Figure 3.23 Oxygen concentration in a $108 \mu\text{m}$ radius pre-antral follicle with various vascular distributions (50% vasc, $\varepsilon = 0.3$). Concentration scales have units of mol.m^{-3} . 90
- Figure 3.24 Oxygen concentration in a $108 \mu\text{m}$ radius pre-antral follicle with various vascular distributions (75% vasc, $\varepsilon = 0.3$). Concentration scales have units of mol.m^{-3} . 91
- Figure 3.25 Mean total follicle oxygen concentration for various vascular distributions. 92
- Figure 3.26 Mean oxygen concentration within the area occupied by the oocyte for various vascular distributions. 93

Figure 4.1	The antral and pre-ovulatory follicles.	102
Figure 4.2	Pictorial representation of the model of oxygen transport in the antral follicle. See nomenclature for symbols.	103
Figure 4.3	Oxygen concentration profiles across a follicle ($r_f=200 \mu\text{m}$) with various antrum sizes (parameters at nominal values).	106
Figure 4.4	Volume of antral fluid and granulosa cells required to maintain oxygenation of the granulosa cell layer as a function of total follicle volume.	108
Figure 4.5	The ratio of antral fluid volume to total follicle volume required to maintain oxygenation of the granulosa cell layer as a function of total follicle volume.	111
Figure 4.6	Changes in human granulosa cell number (a) and antral fluid volume (b) as a function of follicle diameter throughout the early antral, late antral, and pre-ovulatory stages of follicle development (taken from McNatty, 1981).	112
Figure 4.7	Antral and granulosa cell volume increase in the human follicle (data adapted from McNatty, 1981) compared to model calculations for mass transport limited follicle growth.	113
Figure 4.8	V_a/V_f in the human follicle (data adapted from McNatty, 1981) using both high and low granulosa cell volume estimates compared to model calculations for mass transport limited follicle growth.	114
Figure 4.9	Distribution of human IVF antral fluid volumes for samples taken from single follicles.	118
Figure 4.10	Distribution of human IVF antral fluid volumes including samples from multiple follicles.	119
Figure 4.11	V_a/V_f as a function of total follicle volume when the granulosa cell layer is oxygenated at $C \geq 0$ and $C \geq C_{crit}$.	127
Figure 4.12	The effect of reducing mean symmetrical vascularisation on V_a/V_f and its relation to total follicle volume (granulosa cell layer oxygenated at $C \geq 0$).	128

- Figure 4.13 Relationship between follicle vascularisation and V_a/V_f for a large antral follicle ($V_f = 1.5$ ml, granulosa cell layer oxygenated at $C \geq 0$). The effect of an increase in V_a/V_f for a follicle with 70% vascularisation on oxygen status is highlighted. 132
- Figure 4.14 Pictorial representation of the model of oxygen transport in the pre-ovulatory follicle (refer to nomenclature section for symbol meanings). 135
- Figure 4.15 Comparison of oxygen concentration profiles across antral and pre-ovulatory follicles for IVF sized follicles (4 ml antral fluid volume (V_a)). 144
- Figure 4.16 Minimum interfacial oxygen concentration (C_{imin}) required to sustain the oocyte at 99% maximal respiration as a function of follicular fluid volume (V_a). Note that oxygen concentration becomes constant before typical IVF volumes attained. 145
- Figure 4.17 Minimum interfacial oxygen concentration required to sustain oocyte at 99% maximal respiration for antral and pre-ovulatory across a range of follicular fluid volumes typical of IVF. The zone of uncertainty represents the dissolved oxygen range within which the respiratory status of the oocyte will be uncertain. Here this is due the uncertainty in the position of the oocyte. 147
- Figure 4.18 Effect of variability in oocyte radius (r_o) through mean \pm 2SD on minimum interfacial oxygen concentration required to sustain oocyte at 99% maximal respiration (IVF size follicle range). The width of the zone of uncertainty is now determined by positional uncertainty of the oocyte (antral or pre-ovulatory) and variability in parameter estimates (in this case r_o). 148
- Figure 4.19 Effect of variability in follicular fluid oxygen diffusion coefficient (D_a) on the minimum interfacial oxygen concentration required to sustain the oocyte at 99% maximal respiration (IVF size follicle range). 152

Figure 4.20	Effect of variability in both r_o and D_a on the minimum interfacial oxygen concentration required to sustain the oocyte at 99% maximal respiration (IVF size follicle range).	153
Figure 4.21	Minimum % vascularisation required to sustain oocyte at 99% maximal respiration as a function of follicular fluid volume (V_a).	156
Figure 4.22	Pictorial representation of the model of oxygen transport in the pre-ovulatory follicle with cumulus (see nomenclature section for description of symbols).	159
Figure 4.23	Comparison of oxygen concentration profiles across a pre-ovulatory follicle with no cumulus and a pre-ovulatory follicle with cumulus (4 ml antral fluid volume (V_a)).	167
Figure 4.24	Comparison of the minimum interfacial oxygen concentration required to sustain the oocyte at 99% of maximal respiration as a function of follicular fluid volume for the pre-ovulatory follicle with and without cumulus. Note that oxygen concentration becomes constant well before typical IVF volumes are reached.	171
Figure 4.25	Minimum interfacial oxygen concentration required to sustain oocyte at 99% maximal respiration for antral, pre-ovulatory with no cumulus, and pre-ovulatory follicles with cumulus (IVF sized follicle range). These concentrations can once again be used to form a zone of uncertainty.	172
Figure 4.26	Effect of variability in follicular fluid solubility between 50% below and 50% above that of plasma on the zone of uncertainty.	174
Figure 5.1	Schematic of the experimental set-up. Inset shows details of the stainless steel cap which houses the cathode, membrane, sample, and lid with exhaust.	183
Figure 5.2.	An example data trace of normalised current vs. time showing progression through various steady-state and transient periods.	184
Figure 5.3	The dissolved oxygen electrode, without (a), and with (b) a sample solution layer.	187

Figure 5.4	Expected schematic transients for different combinations of boundary and initial conditions: (a) switch on, (b) steady-state sample addition, (c) up-step from $p_g=0$, (d) down-step to $p_g=0$.	191
Figure 5.5.	Schematic representation of the finite difference solution to the model.	198
Figure 5.6	Simulated ‘down-step’ transients under the assumption of non-linear and linear membrane partial pressure profiles at various ratios of L_s/L_m .	204
Figure 5.7	Simulated ‘up-step’ transients under the assumption of non-linear and linear membrane partial pressure profiles at various ratios of L_s/L_m .	205
Figure 5.8	Simulated ‘switch-on’ transients under the assumption of non-linear and linear membrane partial pressure profiles at various ratios of L_s/L_m .	206
Figure 5.9	Simulated ‘steady-state sample addition’ transients under the assumption of non-linear and linear membrane partial pressure profiles at various ratios of L_s/L_m .	207
Figure 5.10	Experimental ‘sample steady-state addition’ traces showing (a) acceptable and (b) discarded experimental data.	212
Figure 5.11	Experimental ‘down-step’ traces showing (a) acceptable and (b) discarded experimental data.	213
Figure 5.12	Algorithm for calculating diffusivity (ideally $x = 0$, however to save processing time x was chosen as 0.001. Precision used here was 0.01×10^{-9}).	214
Figure 5.13	Change in predicted sample diffusion coefficient with time for ‘steady-state sample addition’. Horizontal line indicates standard value for the oxygen diffusion coefficient in water at 25 °C (Lango <i>et al.</i> , 1996).	218
Figure 5.14	Change in predicted sample diffusion coefficient with time for ‘down-step’. Horizontal line indicates standard value for the oxygen diffusion coefficient in water at 25 °C (Lango <i>et al.</i> , 1996).	219

- Figure 6.1 Box plots of the density, osmolality, and viscosity of human follicular fluid samples. Whiskers represent the range (and define scale), while the box represents the upper quartile, median, and lower quartile. Ranges for human plasma obtained from the literature are included for comparison. 229
- Figure 6.2 Box plots of the oxygen diffusivity, solubility, and permeability in human follicular fluid samples. Whiskers represent the range (and define scale), while the box represents the upper quartile, median, and lower quartile. Ranges for human plasma obtained from the literature are included for comparison. 235
- Figure 6.3 Minimum follicular fluid dissolved oxygen required to sustain the oocyte at 99% maximal respiration (IVF sized follicle range). The antral and pre-ovulatory with cumulus models can be used to set the lower and upper bounds of the zone of uncertainty respectively, and reflect the positional uncertainty of the cumulus-oocyte complex. The parameters of oocyte size, follicular fluid diffusivity, and solubility are used in combination to obtain the maximal width of the zone of uncertainty based on knowledge of variability in these parameters (including the measurements of D_a and S_a reported here). 237
- Figure 7.1 Aspiration set-up and measurement sites. 241
- Figure 7.2 A typical bovine cumulus-oocyte complex after maturation in synthetic media. 245
- Figure 7.3 Visual description of various measured parameters used to provide a semi-quantitative description of the cumulus-oocyte complex. Refer to text or nomenclature section for description of symbols. 248
- Figure 7.4 Comparison of the dissolved oxygen in follicular fluid before aspiration with that in the collection vial, or when sampled from tubing line before the collection vial (see position III, Figure 7.1). Symbols: \blacklozenge - fluid in collection tube; \triangle - fluid from position III. Straight line is $y=x$. 251
- Figure 7.5 Mean change in pH of follicular fluid before and after aspiration, for both normal aspiration and when fluid is sampled from the tubing

	line. Error bars show standard deviation.	254
Figure 7.6	Changes in dissolved oxygen of a 5 ml follicular fluid sample with various initial dissolved oxygen levels upon contamination with blood.	259
Figure 7.7	Mean temperature of follicular fluid during aspiration at positions indicated in Figure 7.1.	264
Figure 7.8	Heat loss from the tubing exit to the bottom of the collection vial (T IV to T V) as a function of flow rate.	267
Figure 7.9	Effect of IVF aspiration on the area of cumulus cells surrounding the oocyte.	277
Figure 7.10	Effect of IVF aspiration on the coverage, voidage, and sphericity of cumulus cells	278
Figure 8.1	Suggested alternative approaches to the sampling and/or measurement of dissolved oxygen in follicular fluid.	286
Figure 8.2	In-line sampling devices.	294
Figure 8.3	Dissolved oxygen levels before and after sampling using devices A, B, and C. In each case the solid line is that of $y=x$.	299
Figure 8.4	Bland-Altman Plots for devices A, B and C.	300
Figure 8.5	Box plots of differences (after aspiration-before) for cumulus sphericity, for normal IVF and syringe sampling. Adjacent box plots are on common scale which is defined by the whiskers. Whiskers represent range with the box showing upper and lower quartiles, as well as the median.	304
Figure 8.6	The improved sampling device (taken from Harding, 2005).	306
Figure 8.7	Typical fibrinogen clots before and after aspiration (taken from Harding 2005). Divisions on scale = 1 mm.	308
Figure 8.8	Oxygen before and after aspiration as measured by an in-line fluorescence probe (Collier, personal communication).	310

LIST OF TABLES

Table 2.1	Comparison of reported values of the pH of human follicular fluid.	26
Table 3.1	Parameter values used by Gosden & Byatt-Smith (1986) (* R_g is expressed \pm SD). Right hand column expresses values in units appropriate to the model discussed in section 3.3.1.	45
Table 3.2	Oxygen consumption rates of various human tissues compared to granulosa cells. All consumption rates have units of $\text{mol}\cdot\text{m}^{-3}\cdot\text{s}^{-1}$.	49
Table 3.3	Ranges of interest and nominal values for the pre-antral follicle model.	56
Table 4.1	A comparison of human oocyte oxygen consumption to that of other species. Note that R_o is reported both on a per oocyte basis and after normalisation for oocyte volume of a given species.	123
Table 5.1.	Relative permeability (sample to water) of sucrose and NaCl standards of various concentrations.	190
Table 5.2	Parameter estimates for input into finite difference solutions at 25°C.	201
Table 5.3	Oxygen permeability, diffusivity, and solubility data for ‘sample steady-state addition’ method for various standard solutions (four replicates for each standard). Experimental values of relative solubility are compared to those of MacArthur 1916 by calculating the difference (experimental value minus MacArthur value).	220
Table 5.4	Oxygen permeability, diffusivity, and solubility data for the ‘down-step’ method for various standard solutions (four replicates for each standard). Experimental values of relative solubility are compared to those of MacArthur 1916 by calculating the difference (experimental value minus MacArthur value).	221

Table 6.1	Relative density, osmolality, and relative viscosity in human follicular fluid. Values for human plasma are included for comparison.	227
Table 6.2	Relative diffusivity, relative solubility, and relative permeability of oxygen in human follicular fluid. Values for human plasma are included for comparison.	234
Table 7.1	Variation in reported values of follicular fluid oxygen levels.	252
Table 7.2	Parameter estimates for blood contamination calculations.	258
Table 7.3	Temperature of follicular fluid (°C) at positions indicated in Figure 7.1, for a standard aspiration kit.	263
Table 7.4	Temperature drop from thermocouple IV to V under different heating conditions (°C). $\Delta\theta_1$ is the temperature drop when aspiration is performed normally. $\Delta\theta_2$ is the temperature drop when the collection vial is heated and then insulated prior to aspiration. $\Delta\theta_3$ is the temperature drop when the collection vial is insulated only prior to aspiration.	268
Table 7.5	Summary of paired observations analysis of the effects of IVF aspiration on the cumulus-oocyte complex. All differences are mean values apart from compact and expanded cumulus coverage the values for which represent the increase in the proportion of not fully covered observations. * indicates a significant difference at a minimum of the 5% level.	279

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	$\text{m}^2.\text{s}^{-1}$
D_{cell}	diffusion coefficient of oxygen through the cellular fraction of tissue	$\text{m}^2.\text{s}^{-1}$
D_p	diffusion coefficient of oxygen in plasma	$\text{m}^2.\text{s}^{-1}$
D_{eff}	effective diffusion coefficient of oxygen through tissue	$\text{m}^2.\text{s}^{-1}$
D_a	diffusion coefficient of oxygen in the antral fluid	m
D_c	diffusion coefficient of oxygen in the cumulus cell layer	$\text{m}^2.\text{s}^{-1}$
D_m	diffusivity of oxygen in the membrane of a dissolved oxygen electrode	$\text{m}^2.\text{s}^{-1}$
D_s	diffusivity of oxygen in the sample solution	$\text{m}^2.\text{s}^{-1}$
D_w	diffusivity of oxygen in water	$\text{m}^2.\text{s}^{-1}$
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_{O_2}	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
p_{O_2}	partial pressure of oxygen	mmHg
$p_{O_{2b}}$	partial pressure of oxygen in blood	mmHg
$p_{O_{2f}}$	partial pressure of oxygen in follicular fluid	mmHg
$p_{O_{2m}}$	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	$\text{mol.m}^{-3}.\text{s}^{-1}$
R_g	oxygen consumption rate of the granulosa cells	$\text{mol.m}^{-3}.\text{s}^{-1}$
R_{omax}	maximal oxygen consumption rate by the oocyte	$\text{mol.m}^{-3}.\text{s}^{-1}$
R_o	oxygen consumption rate by the oocyte	$\text{mol.m}^{-3}.\text{s}^{-1}$
R	gas constant	$\text{L.atm.mol}^{-1}.\text{K}^{-1}$
R_a	oxygen consumption rate in the fluid antrum	$\text{mol.m}^{-3}.\text{s}^{-1}$
R_c	oxygen consumption rate of the cumulus cells	$\text{mol.m}^{-3}.\text{s}^{-1}$
S	solubility of oxygen in solution	$\text{mol.m}^{-3}.\text{mmHg}^{-1}$
S_a	solubility of oxygen in the antral fluid	$\text{mol.m}^{-3}.\text{mmHg}^{-1}$
S_m	solubility of oxygen in the membrane of a dissolved oxygen electrode	$\text{mol.m}^{-3}.\text{mmHg}^{-1}$
S_s	solubility of oxygen in the sample solution	$\text{mol.m}^{-3}.\text{mmHg}^{-1}$
S_w	solubility of oxygen in water	$\text{mol.m}^{-3}.\text{mmHg}^{-1}$
S_{rel}	relative solubility of oxygen in sample compared to water	
S_p	solubility of oxygen in plasma	$\text{mol.m}^{-3}.\text{mmHg}^{-1}$
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}
$\%v_{asc}$	mean symmetrical vascularisation	%
V_g	volume of granulosa cells in follicle	m^3
V_{cell}	volume of a single granulosa cell	$\text{m}^3.\text{cell}^{-1}$
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_{O_2}	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	$^{\circ}\text{C}$
$\Delta\theta$	temperature change	$^{\circ}\text{C}$
$\Delta\theta_{tot}$	total temperature change over entire aspiration kit	$^{\circ}\text{C}$
$\Delta\theta_1$	temperature drop over the collection vial when aspiration is performed normally	$^{\circ}\text{C}$

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