

University of Dundee

## MASTER OF SCIENCE

### Investigating the effect of phosphodiesterase inhibitors on human sperm motility and function

Rice, Anne

*Award date:*  
2011

[Link to publication](#)

#### **General rights**

Copyright and moral rights for the publications made accessible in the public portal are retained by the authors and/or other copyright owners and it is a condition of accessing publications that users recognise and abide by the legal requirements associated with these rights.

- Users may download and print one copy of any publication from the public portal for the purpose of private study or research.
- You may not further distribute the material or use it for any profit-making activity or commercial gain
- You may freely distribute the URL identifying the publication in the public portal

#### **Take down policy**

If you believe that this document breaches copyright please contact us providing details, and we will remove access to the work immediately and investigate your claim.

Investigating the effect of  
phosphodiesterase inhibitors on  
human sperm motility and function

A. Rice

MSc

University of Dundee



September 2011

## Contents

Contents .....	II
List of figures .....	VI
List of tables .....	IX
Acknowledgements .....	X
Declaration .....	XI
Abstract .....	XII
Chapter 1: Introduction .....	1
1.1 Development of Assisted Reproductive Technology (ART) .....	1
1.2 Causes of male infertility .....	3
1.3 Sperm motility .....	6
1.3.1 Inhibition of motility .....	8
1.3.2 Stimulation of motility – interfering with cAMP using PDE inhibitors.....	9
1.4 Sperm-oocyte interaction .....	18
1.5 Aims and objectives .....	20
Chapter 2: Investigating the effect of PDE-1 and PDE-4 specific inhibitors on human sperm motility .....	22
2.1 Introduction .....	22
2.2 Materials and Methods .....	24
2.2.1 Sperm preparation.....	25
2.2.2 Sperm-PDE inhibitor incubation .....	27
2.3 Results: Effect of PDE inhibitors on sperm motility.....	32
2.3.1 Effect of PDE inhibitors on ‘healthy’ donor population.....	32
2.3.2 Effect of PDE inhibitors on patient samples.....	36
2.4 Discussion .....	40
Chapter 3: Investigating the effects of PDE inhibitors using different sperm media, PDE inhibitor concentrations and longer incubation periods .....	42
3.1 Investigating the effect of PDE inhibitors using non-capacitating media.....	42

3.1.1 Introduction.....	42
3.1.2 Materials and Methods .....	44
3.1.3 Results .....	44
3.1.4 Discussion .....	46
3.2 Effects of varying incubation periods and concentrations of PDE inhibitor on human sperm motility.....	48
3.2.1 Introduction.....	48
3.2.2 Materials and Methods.....	50
3.2.3 Results.....	51
3.2.4 Discussion.....	60
3.3 The effect of combining PDE inhibitors on human sperm motility .....	62
3.3.1 Introduction.....	62
3.3.2 Materials and Methods.....	63
3.3.3 Results.....	63
3.3.4 Discussion.....	64
3.4 The effect of washing sperm after PDE inhibitor incubation.....	65
3.4.1 Introduction.....	65
3.4.2 Materials and Methods.....	66
3.4.3 Results.....	67
3.4.4 Discussion.....	68
Chapter 4: Sperm physiology and PDE inhibitors .....	70
4.1 Acrosome Reaction .....	70
4.1.1 Introduction.....	70
4.1.2 Materials and Methods.....	73
4.1.3 Results.....	75
4.1.4 Discussion.....	77
4.2 Tunel Assay.....	79

4.2.1 Introduction.....	79
4.2.2 Materials and Methods.....	81
4.2.3 Results.....	82
4.2.4 Discussion.....	84
Chapter 5: Further investigation on the effect of PDE inhibitors on human sperm motility.....	86
5.1 Introduction.....	86
5.2 Materials and Methods.....	87
5.3 Results.....	88
5.4 Discussion.....	97
Chapter 6: Screening patient samples.....	100
6.1 Introduction.....	100
6.2 Materials and Methods.....	101
6.3 Results.....	103
6.4 Discussion.....	109
Chapter 7: Investigating the effects of PDE specific inhibitors on human sperm function.....	112
7.1 Sperm penetration test.....	112
7.1.1 Introduction.....	112
7.1.2 Materials and Methods.....	114
7.1.3 Results.....	116
7.1.4 Discussion.....	118
7.2 Human sperm-zona pellucida binding test.....	119
7.2.1 Introduction.....	119
7.2.2 Materials and Methods.....	123
7.2.3 Results.....	127
7.2.4 Discussion.....	130
Chapter 8: General discussion.....	133

List of references.....	139
Appendix.....	151

## List of Figures

<b>Figures</b>	<b>Page</b>
<b>Figure 1.1</b> ART and ICSI cycles performed 1999-2008 .....	2
<b>Figure 1.2</b> Percent of transfers resulting in live birth.....	3
<b>Figure 1.3</b> Reasons for fertility treatment .....	5
<b>Figure 1.4</b> Percent of transfers from ICSI cycles by reason for infertility.....	6
<b>Figure 1.5</b> Diagram demonstrating cAMP dependent pathway .....	21
<b>Figure 2.1</b> Percent motile cells in 80% fraction of donor sperm with 8-MeOM- IBMX .....	33
<b>Figure 2.2</b> Percent motile cells in 80% fraction donor sperm with Rolipram.....	34
<b>Figure 2.3</b> Percent motile cells in 40% fraction donor sperm with 8-MeOM-IBMX	35
<b>Figure 2.4</b> Percent motile cells in 40% fraction donor sperm with Rolipram.....	36
<b>Figure 2.5</b> Percent motile cells in 40% fraction patient sperm with 8-MeOM- IBMX .....	37
<b>Figure 2.6</b> Percent motile cells in 40% fraction patient sperm with Rolipram.....	38
<b>Figure 3.1.1</b> Percent motile cells in 40% fraction patient sperm with 8-MeOM- IBMX in HEPES media .....	45
<b>Figure 3.1.2</b> Percent motile cells in 40% fraction patient sperm with Rolipram media .....	46
<b>Figure 3.2.1</b> Percent motile cells in 80% fraction donor sperm with 100 $\mu$ M 8- MeOM-IBMX over 8 hours .....	52
<b>Figure 3.2.2</b> Percent motile cells in 80% fraction donor sperm with 200 $\mu$ M 8- MeOM-IBMX over 8 hours. ....	53

<b>Figure 3.2.3</b> Percent motile cells in 40% fraction donor sperm with 100 $\mu$ M 8-MeOM-IBMX over 8 hours. ....	54
<b>Figure 3.2.4</b> Percent motile cells in 40% fraction donor sperm with 200 $\mu$ M 8-MeOM-IBMX over 8 hours .....	55
<b>Figure 3.2.5</b> Percent motile cells in 40% fraction patient sperm with 100 $\mu$ M 8-MeOM-IBMX over 8 hours. ....	57
<b>Figure 3.2.6</b> Percent motile cells in 40% fraction patient sperm with 200 $\mu$ M 8-MeOM-IBMX over 8 hours. ....	58
<b>Figure 3.2.7</b> Percent motile cells in 40% fraction patient sperm with 10 $\mu$ M Rolipram over 8 hours.....	59
<b>Figure 3.3.1</b> Percent motile cells in 40% fraction patient sperm with 8-MeOM-IBMX and Rolipram .....	64
<b>Figure 3.4.1</b> Percent motile cells in 40% fraction patient sperm with either 8-MeOM-IBMX or Rolipram after wash .....	68
<b>Figure 4.1</b> Percent of live cells acrosome intact/reacted after addition of 8-MeOM-IBMX or Rolipram.....	76
<b>Figure 4.2</b> Percent of cells with DNA damage after incubation with 8-MeOM-IBMX or Rolipram .....	83
<b>Figure 5.1</b> Percent motile cells in 40% fraction patient sperm with Milrinone .....	89
<b>Figure 5.2</b> Percent progressive cells in 40% fraction patient sperm with Milrinone.....	90
<b>Figure 5.3</b> Percent motile cells in 40% fraction patient sperm with BRL .....	91
<b>Figure 5.4</b> Percent progressive cells in 40% fraction patient sperm with BRL .....	92
<b>Figure 5.5</b> Percent motile cells in 40% fraction patient sperm with Papaverine .....	93
<b>Figure 5.6</b> Percent progressive cells in 40% fraction patient sperm with	



Papaverine .....	94
<b>Figure 5.7</b> Percent motile cells in 40% fraction patient sperm with BRL after wash .....	96
<b>Figure 6.1</b> Percent motile cells in 80% fraction IVF patient sperm with either Rolipram or BRL .....	104
<b>Figure 6.2</b> Percent motile cells in 80% fraction ICSI patient sperm with either Rolipram or BRL .....	105
<b>Figure 6.3</b> Percent progressive motile cells in 80% fraction IVF patient sperm with Rolipram.....	106
<b>Figure 6.4</b> Percent progressive motile cells in 80% fraction IVF patient sperm with BRL.....	107
<b>Figure 6.5</b> Percent progressive motile cells in 80% fraction ICSI patient sperm with Rolipram.....	108
<b>Figure 6.6</b> Percent progressive motile cells in 80% fraction ICSI patient sperm with BRL.....	109
<b>Figure 7.1.1</b> Sperm penetration test Part 1 .....	116
<b>Figure 7.1.2</b> Sperm penetration test Part 2 .....	117
<b>Figure 7.2.1</b> Sperm-zona pellucida binding .....	122
<b>Figure 7.2.2</b> Dissected zona-pellucida .....	125
<b>Figure 7.2.3</b> Sperm-zona pellucida magnified .....	126
<b>Figure 7.2.4</b> Hemi-zona assay .....	127
<b>Figure 7.2.5</b> Sperm-zona pellucida binding results (1) .....	129
<b>Figure 7.2.6</b> Sperm-zona pellucida binding results (2) .....	129
<b>Figure 7.2.7</b> Graph of sperm-zona pellucida binding results .....	130

<b>List of Tables</b>	<b>Page</b>
<b>Table 2.1</b> PDE Inhibitor concentrations .....	28
<b>Table 2.2</b> Sperm motility parameters .....	29
<b>Table 2.3</b> Effect of 8-MeOM-IBMX on donor/patient sperm.....	39
<b>Table 2.4</b> Effect of Rolipram on donor/patient sperm.....	40
<b>Table 4.1</b> Effect of PDE inhibitors on sperm acrosome.....	77
<b>Table 5.1</b> Comparison of PDE inhibitor effects on sperm motility.....	95
<b>Table 7.1</b> Effect of PDE inhibitor on sperm-zona pellucida binding .....	128
<b>Table 8.1</b> Clinical effect of PTX .....	138

## **Acknowledgements**

I would like to offer my most sincere gratitude to all the people who have helped me over the past two years. This includes everyone in the MACHS labs and the embryology team of Ninewells Hospital's ACU.

I would like to thank Christopher Barratt for his enthusiasm, inspiration and support and Evelyn Barratt for her great efforts in consenting patients for this thesis.

I would especially like to thank Lead Clinical Embryologist Katherine Whalley for sharing lots of good ideas and allowing me to use the clinic's laboratory and equipment for this thesis. Her encouragement and advice, (not to mention patience!) is greatly appreciated.

I also thank my Gran, my friends and Michael Quinn for all the support throughout the two years. Without their support this thesis would not have been possible.

**Declaration**

The data used in this thesis was gathered by me alone and I am the sole author of the text. I have personally cited all references and have not submitted this thesis previously for any other degree.

Name     Anne Rice

Signed    .....

**Abstract**

Assisted reproductive technologies (ART) have rapidly progressed through the years and it is becoming increasingly apparent that the main cause of infertility is male factor. Today, ICSI represents most treatment which itself demonstrates the severity of male infertility. This may include problems with sperm concentration, motility and/or morphology. Sperm motility defects are associated with most cases of male infertility and provide problems for sperm reaching, binding to and penetrating the oocyte therefore impacting on fertilisation success.

Improving motility of human sperm has previously been investigated using various PDE inhibitors. PDE inhibitors have been shown to enhance sperm motility and studies have associated this with predicting an increase in IVF rates. Enhancing sperm motility increases the probability of a successful result. With the use of PDE inhibitors it may also be possible to improve sperm function. This includes enabling sperm to bind to the zona-pellucida of human oocytes, a technique that is closely correlated with predicting IVF outcome.

This study investigates the effect of various PDE inhibitors on human sperm motility of samples from both fertile and sub-fertile men. This is done using CASA throughout to record motile and progressively motile cells. Experiments were carried out on various PDE inhibitors, some of which have previously been reported as having an effect on sperm motility and some which have never been investigated before. The study then goes on to investigate the effect of PDE inhibitors on sperm function of samples from sub-fertile men. This includes a sperm penetration test to

determine whether PDE inhibitors allow sperm to penetrate a viscous substance more easily, mimicking the *in-vivo* action of penetrating cervical mucus in the female reproductive tract. Lastly, this study explores the effect of PDE inhibitors on the ability of sperm from sub-fertile men to bind to the zona-pellucida of human oocytes. This study is a novel approach in the clinical investigation of PDE inhibitors in the human sperm-oocyte interaction stage of fertilisation.