



**The Effect of Cytokines  
on  
Chorionic Gonadotrophin  
Expression  
in the  
Marmoset Monkey Embryo**

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## Table of Contents

Table of Contents	i
Summary	iii
Declaration	vi
Acknowledgments	vii
Publications	x
Conference Presentations	xi
Awards	xiii
List of Abbreviations used in this Thesis	xiv
Chapter One: Review of the Literature	
1.1 Introduction	1
1.2 Early Embryonic Development	2
1.3 Maternal Recognition of Pregnancy	10
1.4 Cytokines and Growth Factors	26
1.5 <i>In Vitro</i> Culture of Pre-implantation Embryos	51
1.6 Hypothesis and Aims of the Thesis	56
Chapter Two: Materials and Methods	
2.1 Reagents and Solutions	57
2.2 Animals	58
2.3 Culture of Marmoset Trophoblastic Vesicles and Marmoset Embryos	62
2.4 RNA Extraction	65
2.5 Reverse Transcriptase-Polymerase Chain Reaction (RT-PCR) Protocols	67
2.6 Cloning of DNA Fragments	85
2.7 ELISA	91
Chapter Three: Qualitative RT-PCR	
3.1 Introduction	95
3.2 Collection of Embryos and Other Tissues	96
3.3 RNA Extraction of Embryos and Other Tissues	97
3.4 Qualitative RT-PCR	99
3.5 Results	107
3.6 Discussion	110

Chapter Four: Quantitative RT-PCR	
4.1 Introduction	114
4.2 Construction of Internal Standards	116
4.3 Development of Quantitative Assays	123
4.3.6 Results	128
4.5 Discussion	143
Chapter Five: ELISA	
5.1 Introduction	148
5.2 Materials and Methods	150
5.3 Results	152
5.5 Discussion	166
Chapter Six: Culture of Marmoset Trophoblastic Vesicles and Embryos with Cytokines	
6.1 Introduction	170
6.2 Collection of Embryos	172
6.3 Culture of Embryos through to Trophoblastic Vesicles	173
6.4 Culture of Trophoblastic Vesicles and Fragments with Cytokines	174
6.5 Culture of Embryos with Cytokines	187
6.7 Discussion	200
Chapter Seven: Final Discussion	208
Bibliography	216
Appendix	243

## **Summary**

Chorionic gonadotrophin (CG) is one of the first factors produced by the embryo to signal its presence to the mother and thus prevent atrophy of the corpus luteum, thereby ensuring the continued production of progesterone to favour embryonic development. CG, a primate specific glycoprotein hormone, is produced by the trophoblast and is first detected in the human peripheral blood between Days 8-11 after ovulation. However, *in vitro* studies have shown that CG is secreted by the embryo before it can be detected in the peripheral blood.

Cytokines and growth factors are a group of proteins and polypeptides, released from the cell, with a variety of functions, including intracellular communication and alteration and regulation of cell growth and differentiation. Various cytokines and growth factors are involved in the ovulatory process, in the immune response between mother and embryo, as well as in the implantation process. A variety of cytokines have been shown to modify CG expression in human tumour cell lines, modify differentiation of the embryonic cells and to be involved in communication between the mother and the embryo. Various cytokine ligands and receptors for these factors have been found to be expressed by the embryo.

The characterisation of the role of cytokines and growth factors has been conducted predominantly on rodents, with few results attained using primate tissue or embryos. Hence, to

determine the involvement of these factors in the regulation of CG expression, we have used the Common Marmoset (*Callithrix jacchus*) as a model primate.

The purpose of this project was to examine the cellular and molecular processes involved in expression of embryonic signals and the initial interactions that occur between the embryo and the maternal endometrium of the primate. This knowledge will assist in developing culture conditions for improved viability of human IVF embryos, as well as enhancing our understanding of primate reproductive physiology.

Expression of marmoset CG genes (alpha and beta subunits) was confirmed in pre-implantation marmoset embryos from the eight-cell stage through to the hatching blastocyst stage of development. A method for quantitating marmoset CG- $\beta$  mRNA expression in marmoset tissue, using competitive reverse transcriptase polymerase chain reaction (RT-PCR) was also developed. Marmoset glyceraldehyde-phosphate-dehydrogenase (mGAPDH) was cloned and partially sequenced to use as an endogenous internal standard to compare tissue types and to confirm the success of the reverse transcription reaction.

An enzyme-linked immunosorbent assay (ELISA) was developed, using an inhouse polyclonal antibody raised to recombinant mCG and purified in our laboratory, to measure the amount of mCG present in plasma of cycling and pregnant

marmoset monkeys and the amount of mCG secreted by marmoset trophoblastic vesicles and embryos.

*In vitro* culture of marmoset trophoblastic vesicles was used to study the influence of two cytokines, LIF & GM-CSF, known to be present in the uterine milieu at peri-implantation. LIF is known to regulate differentiation of cytotrophoblasts to anchoring trophoblasts and thus presumably decrease CG expression, whereas GM-CSF is known to enhance CG expression. This culture system was then applied to whole marmoset embryos, in an attempt to gain an insight into primate pre-implantation embryo development and the role these two factors play at implantation. Preliminary results suggest that both cytokines promote blastocyst hatching and attachment. However, more embryos are needed to further quantitate mCG- $\beta$  expression using the competitive PCR assay and to fully understand the full impact of these two factors at implantation.