



**CRYOPRESERVATION AND DEVELOPMENT OF
THE MAMMALIAN OOCYTE**

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ABSTRACT

A successful means of storing the female gamete has yet to be established. The experiments reported in this thesis have been designed to determine methods for preserving mature oocytes and primary ovarian follicles.

Mature ovulated oocytes were frozen using a slow cool to -40°C and rapid thaw technique using DMSO as cryoprotectant. After freezing and thawing the rate of fertilization decreased by about 50% and the occurrence of polyploidy increased by 10%. These results were similar to those previously obtained using different freezing protocols. Further investigations into these problems demonstrated that; (i) the increased rate of polyploidy was due to a reduced ability of frozen-thawed oocytes to extrude the second polar body, (ii) freezing induced changes in the zona pellucida inhibited sperm penetration resulting in a reduced rate of fertilization, (iii) the addition of serum to the freezing medium prevented the changes in the zona pellucida which inhibited sperm penetration, and (iv) the developmental capacity in vitro and in vivo of embryos derived from frozen-thawed oocytes was similar to unfrozen controls.

Previously, oocyte freezing has been associated with a considerable wastage of potentially viable gametes. With the observations in this study a method has been developed in which the overall developmental capacity of frozen-thawed oocytes is similar to unfrozen controls.

As an alternative to the low temperature storage of mature ovulated oocytes, isolated primary ovarian follicles were frozen using a procedure similar to that used for the mature oocyte. After thawing, about 80% of primary follicles were morphologically normal. Fresh and frozen-thawed primary follicles were developed in an extra-ovarian culture system consisting of 6 days in vitro followed by 10 days beneath the kidney capsule of ovariectomized recipients. Similar proportions of fresh and frozen-thawed primary follicles produced mature oocytes. After fertilization in vitro and transfer of 2-cell embryos derived from fresh and frozen-thawed primary follicles, live young were obtained. This demonstrated for the first time the feasibility of preserving the large pool of primary follicles as a source of potentially viable gametes for future use.

In an attempt to simplify the system for extra-ovarian development fresh and frozen-thawed primary follicles were cultured in vitro for 12 days. No difference in either oocyte growth or the acquisition of meiotic competence was observed but the overall recovery of mature oocytes was low (10%). The addition of dbcAMP to the culture medium significantly increased granulosa cell numbers as well as oocyte growth and the proportion of oocytes competent to resume meiosis at the end of the culture period (47%). Further experiments designed to investigate the interaction between granulosa cell function and oocyte growth and development may provide efficient culture systems for the development of fresh and frozen-thawed primary ovarian follicles.

TABLE OF CONTENTS

Title Page	i
Declaration	ii
Acknowledgements	iii
Publications	iv
Abstract	v
Table of contents	vii
GENERAL INTRODUCTION	1
Section 1. REVIEW OF THE LITERATURE	2
1.1 DEVELOPMENT OF THE FEMALE GAMETE	2
1.1.1 Introduction	2
1.1.2 From Primordial Germ Cells to Primordial Follicles	2
1.1.3 From Primordial Follicles to Ovulation	7
1.1.3.1 Folliculogenesis	8
1.1.3.2 Atresia	12
1.1.3.3 Oocyte Growth	13
1.1.3.4 Meiotic Maturation	17
1.1.3.5 Summary - A View to Cryopreservation	24

1.2	PRINCIPLES OF CRYOPRESERVATION	26
1.2.1	Introduction	26
1.2.2	Addition of Cryoprotectants	26
1.2.3	Seeding	28
1.2.4	Freezing	28
1.2.5	Thawing	30
1.2.6	Storage Temperature	31
1.2.7	Rapid Methods of Cryopreservation	32
1.2.8	Summary	32
1.3	CRYOPRESERVATION OF THE FEMALE GAMETE	34
1.3.1	Introduction	34
1.3.2	Early Attempts to Freeze the Mammalian Oocyte and Embryo	35
1.3.3	Successful Freezing and Thawing of Mammalian Oocytes	38
1.3.4	Problems Associated with Oocyte Cryopreservation	39
1.3.5	Cryopreservation of the Human Oocyte	41
1.4	Conclusions - Aims of Thesis	43

Section 2.	CRYOPRESERVATION AND DEVELOPMENT	
	OF MATURE MAMMALIAN OOCYTES	44
2.1	Introduction	44
2.2	Materials & Methods	45
2.3	Assessment of Fertilization After	
	Freezing and Thawing of Mouse Oocytes	50
2.3.1	Introduction	50
2.3.2	Experimental Methods	50
2.3.3	Results	51
2.3.4	Discussion	58
2.4	Investigations Relating to the Decreased	
	Rate of Fertilization of Frozen-Thawed Oocytes	63
2.4.1	Introduction	63
2.4.2	Experimental Methods	64
2.4.3	Results	66
2.4.4	Discussion	69
2.5	The Role of Macromolecules During	
	Oocyte Cryopreservation	75
2.5.1	Introduction	75
2.5.2	Experimental Methods	76
2.5.3	Results	77
2.5.4	Discussion	83

Section 3	CRYOPRESERVATION AND DEVELOPMENT OF MOUSE PRIMARY OVARIAN FOLLICLES	89
3.1	Introduction	89
3.2	Materials & Methods	90
3.3	Extra Ovarian Production of Mature Viable Mouse Oocytes from Frozen Primary Follicles	94
3.3.1	Introduction	94
3.3.2	Experimental Methods	94
3.3.3	Results	95
3.3.4	Discussion	100
3.4	<u>In Vitro</u> Development of Fresh & Frozen-Thawed Primary Ovarian Follicles	108
3.4.1	Introduction	108
3.4.2	Experimental Methods	109
3.4.3	Results	109
3.4.4	Discussion	114

3.5	The Effects of dbcAMP and Hypoxanthine on the Development of Primary Ovarian Follicles Cultured in Collagen Gels	120
3.5.1	Introduction	120
3.5.2	Experimental Methods	121
3.5.3	Results	123
3.5.4	Discussion	130
3.6	Growth and Development of Isolated Mouse Primary Ovarian Follicles in Different Gonadotrophin Environments	138
3.6.1	Introduction	138
3.6.2	Experimental Methods	140
3.6.3	Results	141
3.6.4	Discussion	148
Section 4	CONCLUSIONS	153
	References	159