

CONTENTS

PAGE NUMBER

Acknowledgement	i
List of Contents	ii-vii
List of Tables	viii
Photographs	ix
List of Figures	ix
Graphs	ix
Abstract	x-xi
Abbreviations	xii
1. Introduction	1
2. Literature Review	5
2.1 Oogenesis	5
2.1.1 Maturation of oocytes before birth	5
2.1.2 Maturation of oocytes at puberty	6
2.2 Folliculogenesis and oocyte maturation	8
2.3 Reproductive hormones in the female	9
2.4 Accessory cells and tissues of the oocyte	13
2.5 Ovulation	14
2.5.1 Polar bodies	15
2.6 Spermatogenesis	17
2.7 Sperm transport	18

2.19	The effects of freezing and thawing on oocytes, zona, sperm entry, phases of fertilization and early embryo development	43
2.20	Superovulation and oocyte recovery in mice	45
2.21	Oocyte assessment	46
3.	Objective	46
4	Materials and Methods	47
4.1.1	Mice used for experiments	47
4.1.2	Locally designed 5% Carbon dioxide modular incubator	48
4.1.3	Super ovulation	49
4.1.4	Freezing method	52
4.1.4.1	The newly developed manual freezing technique	54
4.1.5	Thawing method	55
4.1.6	Sperm preparation	55
4.1.7	Insemination	56
4.1.8	Evaluation of cryosurvival after thawing and insemination	57
4.1.9	Assesement of fertilization	57
4.1.10	General Description of experiments	59
4.2	Experiments 1- 4	
	The effects of varying concentrations of sucrose with 3.5 M DMSO in M ₂ medium	61
4.3	Experiments 5-8	
	The effects of varying concentrations of sucrose with 1.5 M DMSO in M ₂ Medium	63
4.4	Experiments 9 -12	
	The effects of 43% of varying concentrations of sucrose with 57% of 1.5 M DMSO in M ₂ medium	64

4.5 Experiments 13-16	
The effects of 14% of varying concentrations of sucrose with 28.5% of 1.5 M DMSO, 28.5% of 1.5M EG & 28.5% of 1.5M Glycerol in M ₂ medium	65
4.6 Experiments 17 -20	
The effects of 0.05 M sucrose with varying concentrations of DMSO in M ₂ medium	66
4.7 Experiments 21-24	
The effects of 43% of varying concentrations of sucrose with 57% of 1.5 M glycerol in M ₂ medium	67
4.8 Experiments 25-28	
The effects of 0.2M sucrose with different concentrations of glycerol in M ₂ medium	68
4.9 Experiments 29-32	
The effects of 43% of varying concentrations of sucrose with 57% of 1.5M EG in M ₂ medium	69
4.10 Experiments 33-34	
The effects of two concentrations of sucrose with 1.5M EG in M ₂ medium	70
4.11 Experiment 35	
The effect of 20% EG, 1.5M DMSO & 10% human serum in PBS with no sucrose	71
4.12 Experiment 36	
The effect of 0.2M sucrose with 1.5M EG, & 20% human serum in PBS	72
4.13 Experiment 37	
The effect of 0.2M sucrose with 1.5M DMSO, & 20% human serum in EBSS	73

4.14 Experiments 38-39	
The effect of Sperm freeze and embryo freeze	74
4.15 Experiments 40-43	
The effects of varying concentrations of sucrose with 1.5 M 1-2 PROH in M ₂ medium	76
4.16 Control experiment	77
5 Results	78
5.1 Control experiment (Direct insemination without freezing)	78
5.2 Experiments 1 - 4	
The effects of varying concentrations of sucrose with 3.5 M DMSO in M ₂ medium.	79
5.3 Experiments 5-8	
The effects of varying concentrations of sucrose with 1.5 M DMSO in M ₂ medium	80
5.4 Experiments 9 -12	
The effects of 43% of varying concentrations of sucrose with 57% of 1.5 M DMSO in M ₂ medium	81
5.5 Experiments 13-16	
The effects of 14% of varying concentrations of sucrose with 28.5% of 1.5 M DMSO, 28.5% of 1.5M EG & 28.5% of 1.5M Glycerol in M ₂ medium	82
5.6 Experiments 17 -20	
The effects of 0.05 M sucrose with varying concentrations of DMSO in M ₂ medium	83
5.7 Experiments 21-24	
The effects of 43% of varying concentrations of sucrose with 57% of 1.5 M glycerol in M ₂ medium	84

5.8 Experiments 25-28	
The effects of 0.2M sucrose with different concentrations of glycerol in M ₂ medium	85
5.9 Experiments 29-32	
The effects of 43% of varying concentrations of sucrose with 57% of 1.5M EG in M ₂ medium	86
5.10 Experiments 33-34	
The effects of two concentrations of sucrose with 1.5M EG in M ₂ medium	87
5.11 Experiment 35	
The effect of 20% EG, 1.5M DMSO & 10% human serum in PBS with no sucrose	87
5.12 Experiment 36	
The effect of 0.2M sucrose with 1.5M EG, & 20% human serum in PBS	88
5.13 Experiment 37	
The effect of 0.2M sucrose with 1.5M DMSO, & 20% human serum in EBSS	88
5.14 Experiments 38-39	
The effect of Sperm Freeze and Embryo Freeze	89
5.15 Experiments 40-43	
The effects of varying concentrations of sucrose with 1.5 M 1-2 PROH in M ₂ medium	89
6. Discussion	90
7. Conclusions	95
8. Recommendations	96
9. References	97
10. Annexure	108

Tables

Table 1: Protocol for experiments 1-4 using varying concentrations of sucrose with 3.5M DMSO	62
Table 2: Protocol for experiments 5-8 using varying concentrations of sucrose with 1.5 M DMSO	63
Table 3: Protocol for experiments 9-12 using 43% of varying concentrations of sucrose with 57% of 1.5 M DMSO	64
Table 4: Protocol for experiments 13-16 using 14% of varying concentrations of sucrose with 28.5% of 1.5 M DMSO, 28.5% of 1.5M EG & 28.5% of 1.5M Glycerol	65
Table 5: Protocol for experiments 17-20 using 0.05M sucrose with varying concentrations of DMSO	66
Table 6: Protocol for experiments 21-24 using 43% of varying concentrations of sucrose with 57% of 1.5M glycerol	67
Table 7: Protocol for experiments 25-28 using 0.2M sucrose with varying concentrations of glycerol	68
Table 8: Protocol for experiments 29-32 using 43% of varying concentrations of sucrose with 57% of 1.5M EG	69
Table 9: Protocol for experiments 33-34 using two concentrations of sucrose with 1.5M EG in M ₂ medium	70
Table 10: Protocol for experiment 35 using 20% EG , 1.5M DMSO and 10% human serum in PBS with no sucrose	71
Table 11: Protocol for experiment 36 using 0.2M sucrose with 1.5M EG, & 20% human serum in PBS	72
Table 12: Protocol for experiment 37 using 0.2M sucrose with 1.5M DMSO & 20% human serum in EBSS	73
Table 13: Protocol for experiment 38 using Sperm freeze	74
Table 14: Protocol for experiment 39 using Embryo freeze	75
Table 15 : Protocol for experiments 40-43 using varying concentrations of sucrose with 1.5 M 1-2 PROH	76

Photographs

4.1.1. Mice used for experiments	47
4.1.2. Locally designed 5% Carbon dioxide incubator used in the experiments	48
4.1.3.1. Paillets and visotubes used for oocyte freezing	50
4.1.3.2. Goblet being inserted into a Liquid Nitrogen dewar in the cryobank	50
4.1.3.3. Freezing tanks (dewars) in the cryobank	51
4.1.3.4. Oocyte with cumulus masses (before fertilization)	51
4.1.9.1. Sperms are penetrating the zona pellucida	58
4.1.9.2. After fertilization (1 st and 2 nd PB)	58

Figures

Fig 1: Oogenesis: one oogonium producing one mature haploid secondary oocyte (ovum)	8
Fig 2: Feedback regulation of ovarian function	9
Fig 3: The hormonal changes in normal menstrual cycle	12
Fig 4: Relation of fimbriae and ovary. Fimbriae collect the oocyte and sweep it into the uterine tube	13
Fig 5: Polar bodies	15
Fig 6: Spermatogenesis: one spermatogonium divides into four haploid mature spermatozoa	17
Fig 7: The phases of fertilization	22

Graphs

2.18.1.2	A programmable freezer technique	40
4.1.3.1	The manual freezing technique	54