

**CONTENTS**

I. INTRODUCTION..... 1

    I.1. In vivo reprogramming ..... 3

        I.1.1. Reprogramming during the development ..... 3

            I.1.1.1. Reprogramming in the germ line ..... 3

                ➤ Genomic imprinting ..... 3

                ➤ Chromatin dynamics during germ cell epigenetic reprogramming..... 4

                ➤ Acquisition of imprints..... 5

            I.1.1.2. Reprogramming during early development..... 8

                ➤ Epigenetic status of the gametes..... 8

                ➤ Epigenetic reprogramming phase I..... 10

                ➤ Epigenetic status of the zygote..... 11

                ➤ Epigenetic reprogramming phase II..... 12

        I.1.2. Reprogramming during in vivo regeneration..... 13

            I.1.2.1. Regeneration ability ..... 13

            I.1.2.2. Epimorphic regeneration ..... 14

            I.1.2.3. Origin of the blastema in limbs and fins regeneration ..... 15

                ➤ Blastema potency ..... 17

            I.1.2.4. Common mechanism in molecular regulation of regeneration..... 17

    I.2. *In vitro* reprogramming..... 20

        I.2.1. Molecular circuitry of pluripotency ..... 20

            I.2.1.1. Pluripotent cells in culture ..... 20

I.2.1.2. Transcription factors that regulate pluripotency.....	21
➤ Oct3/4 .....	21
➤ Sox2.....	21
➤ C-Myc .....	22
➤ Klf4 .....	22
I.2.1.3. Autorregulatory circuitry of pluripotency.....	23
➤ Genetic regulation .....	23
➤ Epigenetic regulation .....	24
➤ Linking the genetic and epigenetic systems .....	27
I.2.2. Strategies of somatic cell reprogramming.....	30
I.2.2.1. Reprogramming by cellular extracts .....	30
I.2.2.2. Reprogramming by pluripotent cell fusion .....	31
I.2.2.3. Reprogramming by somatic cell nuclear transfer.....	32
➤ Reprogramming mechanisms after SCNT .....	32
○ <i>Active demethylation</i> .....	32
○ <i>Passive demethylation</i> .....	33
○ <i>Chromatin remodelling</i> .....	33
➤ Consequences of aberrant epigenetic reprogramming.....	35
A. Aberrant demethylation patterns .....	35
B. Epigenetic memory.....	36
C. X-chromosome-linked development and gene expression.....	38
○ X-chromosome inactivation in mammals.....	38

○ Effects of SCNT on X-chromosome-linked mRNA expression .....	39
D. Telomere length and somatic cloning .....	40
I.2.2.4. Reprogramming by ectopic expression of transcription factors:	
induced Pluripotential Stem Cells (iPS cells) .....	41
➤ Improving iPS cells obtaining .....	41
➤ Which techniques can be regarded as alternatives? .....	44
➤ Sequential expression of pluripotent markers. ....	45
I.2.2.5. Differences in the mechanisms of reprogramming .....	47
I.3. Cell reprogramming in biomedicine. ....	49
I.3.1. Regenerative medicine .....	49
I.3.1.1. Cell therapy .....	49
➤ Transplantation of human Embryonic Stem cells (hES cells).....	49
○ hES cells from embryos produced by nuclear transfer:	
Therapeutic cloning .....	50
○ hES cells from interspecific somatic cell nuclear transfer ...	51
➤ Transplantation of adult stem cells (AS cells) .....	51
○ Stem cell niche and cellular plasticity.....	52
➤ Transplantation of induced pluripotent cells (iPS cells) .....	53
○ A critical point of view .....	53
○ What to do with stem cells research? .....	54

○ Therapeutic potential of iPS cells .....	55
▪ iPS cells: more breakthroughs wanted.....	56
1.3.1.2. Endogenous regeneration.....	58
➤ Endogenous regeneration studies in zebrafish and other low vertebrates.....	59
○ Overexpression of signaling molecules can improve regeneration .....	61
○ Interference with signaling molecules can augment regeneration .....	62
1.3.2. Cancer research.....	63
1.3.2.1. Treatment with mutagens.....	64
1.3.2.2. Mutants from forward genetic screens .....	65
1.3.2.3. Reverse Genetics: Target-Selected Inactivation of Tumor Suppressor .....	65
1.3.2.4. Transgenic Zebrafish Expressing Mammalian Oncogenes.....	66
1.3.2.5. Transplantation of Mammalian Cancer Cells into Zebrafish.....	67
1.3.3. Human disease studies: triggering, evolution and pharmaceutical research .....	68
1.4. Nuclear reprogramming in zebrafish. Technical aspects.....	73
1.4.1. Nuclear transplant.....	73
1.4.1.1. Donor nucleus somatic cells.....	73
➤ Donor cell types .....	73

➤ Somatic cell culture .....	73
○ Cell obtaining.....	73
○ Cell culture.....	73
1.4.1.2. Recipient.....	74
➤ Unfertilized oocytes obtaining .....	74
➤ Characteristics of non activated oocytes.....	74
➤ Oocyte activation.....	75
➤ Dechoriation .....	76
➤ Enucleation .....	76
1.4.1.3. Nuclear transfer technology.....	77
➤ Micromanipulation media .....	77
➤ Nuclear microinjection .....	77
1.4.1.4. Embryo culture .....	78
1.4.2. Germ-line chimaerism.....	79
1.4.2.1. Donor cells.....	79
1.4.2.2. Recipient embryo .....	79
➤ Characteristics of the recipient embryo.....	79
➤ Recipient embryo germ-line penalizing.....	79
1.4.2.3. Chimaerism technology.....	80
➤ Osmolarity media .....	80
➤ Cell injection .....	80
1.4.2.4. Embryo culture .....	81

---

II. OBJECTIVES .....	83
III. EXPERIMENTAL PLANNING .....	84
IV. STUDIES .....	86
STUDY I. Ultraviolet radiation and handling medium osmolarity affect chimaerism success in zebrafish.....	86
STUDY II. Evaluation of presumptive caudal fin blastema cells as candidate donors in intraspecies zebrafish ( <i>Danio rerio</i> ) chimaeras .....	94
STUDY III. Definition of three somatic adult cell nuclear transplant methods in zebrafish ( <i>Danio rerio</i> ): before, during and after egg activation by sperm fertilization .....	102
STUDY IV. Transplant of adult fibroblast nuclei into the central region of metaphase II eggs resulted in mid blastula transition (MBT) embryos .....	114
STUDY V. Electroactivation of zebrafish ( <i>Danio rerio</i> ) eggs.....	123
STUDY VI. Comparison of different activation stimuli efficiency in zebrafish nuclear transplant.....	135
STUDY VII. Reconstruction of heteroparental gynogenetic diploid condition by nuclear transplant in zebrafish: preliminary results.....	144
V. CONCLUSIONS .....	151
VI. IMPLICATIONS .....	153
REFERENCES.....	155