



# ***In vitro* differentiation of mouse stem cells into insulin-secreting cells for diabetes therapy**

A thesis submitted for the degree of Ph.D. of science in zoology  
(Embryology)

By

**Yasmine Hosny Hassan Abd El-Kader**

M. Sc. in Zoology  
(Embryology 2013)

Supervisors

**Late Prof. Dr. Fawzy Ibrahim Amer**

Professor of Vertebrates and Embryology, Department of Zoology,  
Faculty of Science, Ain Shams University

**Prof. Dr. Hamza Ahmed El Shabaka**

Professor of Vertebrates and Embryology, Department of Zoology,  
Faculty of Science, Ain Shams University

**Prof. Dr. Ali Mohamed Ali Abd El- Aal**

Professor of Physiology, Department of Zoology, Faculty of Science,  
Ain Shams University

**Prof. Dr. Ashraf Abdel Halim El-Sayed**

Professor of Physiology, Faculty of Agriculture,  
Cairo University

**Faculty of Science  
Ain Shams University  
2019**



# Acknowledgements

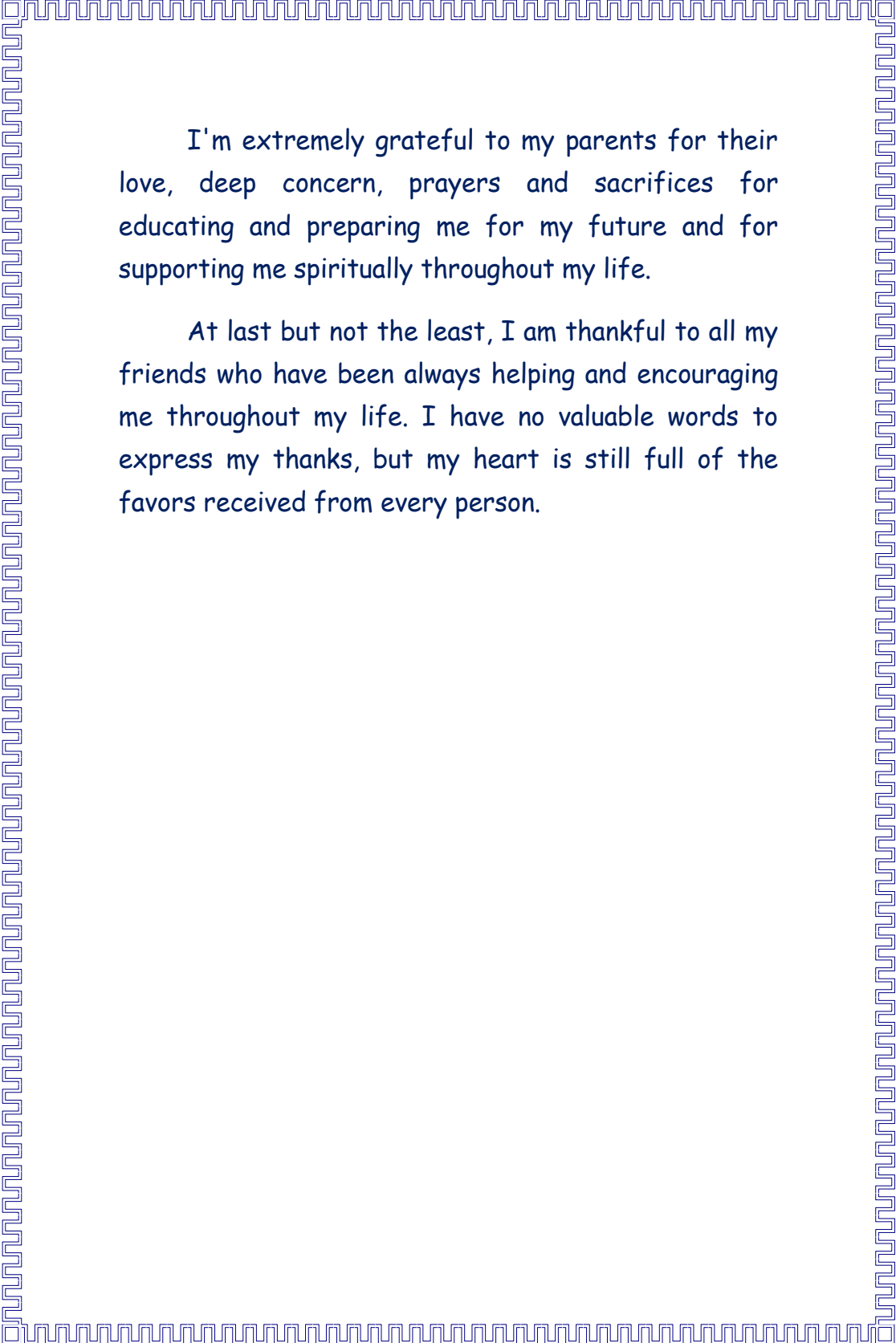
Foremost, I would like to express my love and gratitude to my late supervisor Prof. Dr. Fawzy Amer may God blesses his soul.

I would like to express my sincere gratitude to my advisor Prof. Dr. Hamza El-shabaka for the continuous support of my Ph.D. study and research, for his patience, motivation, enthusiasm, and immense knowledge. His guidance helped me all the time of research and writing of this thesis. I could not have imagined having a better advisor and mentor like him.

I am very thankful to Prof. Dr. Ali Abd El-Aal whose valuable guidance, suggestions and very constructive criticism have contributed immensely throughout my thesis.

Furthermore I would like to thank Prof. Dr. Ashraf El-Sayed for providing the technical facilities throughout my thesis.

Special thanks go to my precious friends Dr. Amr Bayoumy, Dr. Mahmoud Moustafa and Dr. Mohamed Khalifa for their continuous support and the keen interest shown to complete this thesis successfully.



I'm extremely grateful to my parents for their love, deep concern, prayers and sacrifices for educating and preparing me for my future and for supporting me spiritually throughout my life.

At last but not the least, I am thankful to all my friends who have been always helping and encouraging me throughout my life. I have no valuable words to express my thanks, but my heart is still full of the favors received from every person.

| <b>List of Contents</b>                                | <b>Page</b> |
|--------------------------------------------------------|-------------|
| <b>LIST OF ABBREVIATIONS.....</b>                      | <b>I</b>    |
| <b>LIST OF FIGURES .....</b>                           | <b>V</b>    |
| <b>LIST OF TABLES .....</b>                            | <b>XVII</b> |
| <b>INTRODUCTION AND AIM OF THE WORK.....</b>           | <b>1</b>    |
| <b>REVIEW OF LITERATURE .....</b>                      | <b>8</b>    |
| <b>MATERIALS AND METHODS .....</b>                     | <b>37</b>   |
| <b>RESULTS.....</b>                                    | <b>58</b>   |
| <b>• Mesenchymal stem cells.....</b>                   | <b>58</b>   |
| <b>• Histological and ultrastructural studies.....</b> | <b>68</b>   |
| <b>1- The Pancreas.....</b>                            | <b>68</b>   |
| <b>2- The Liver.....</b>                               | <b>93</b>   |
| <b>• Physiological studies.....</b>                    | <b>135</b>  |
| <b>DISSCUSSION.....</b>                                | <b>162</b>  |
| <b>SUMMARY AND CONCLUSIONS .....</b>                   | <b>182</b>  |
| <b>REFERENCES .....</b>                                | <b>186</b>  |



## **LIST OF ABBREVIATIONS**

|        |                                 |
|--------|---------------------------------|
| AC     | Acinus                          |
| ADMSCs | Adipose mesenchymal stem cells  |
| BV     | Blood vessel                    |
| CAT    | Catalase                        |
| CD     | Cluster of differentiation      |
| CV     | Central vein                    |
| DC     | Dark cell                       |
| DM     | Diabetes mellitus               |
| DMEM   | Dulbecco's modified eagle media |
| DTZ    | Dithizone                       |
| EDTA   | Ethylenediaminetetraacetic acid |
| EN     | Endothelial cell                |
| ESCs   | Embryonic stem cells            |
|        |                                 |

---

**LIST OF ABBREVIATIONS**

---

|        |                                              |
|--------|----------------------------------------------|
| FBS    | Fetal bovine serum                           |
| GA     | Golgi apparatus                              |
| GAPDH  | Glyceraldehyde 3-phosphate dehydrogenase     |
| Glut-4 | Glucose transporter-4                        |
| GSH    | Glutathione                                  |
| H      | Hepatocyte                                   |
| H-DMEM | High glucose Dulbecco's modified eagle media |
| HFMSCs | Human fetal mesenchymal stem cells           |
| HPSCs  | Human pluripotent stem cells                 |
| HX-E   | Hematoxylin-Eosin                            |
| IDDM   | Insulin dependent diabetes mellitus          |
| IL     | Islet of Langerhans                          |
| IPCs   | Insulin producing cells                      |
| IPSCs  | Induced pluripotent stem cells               |
| KC     | Kupffer cell                                 |

---

**LIST OF ABBREVIATIONS**

---

|        |                                             |
|--------|---------------------------------------------|
| LC     | Light cell                                  |
| LD     | Lipid droplet                               |
| L-DMEM | Low glucose Dulbecco's modified eagle media |
| Ly     | Lysosome                                    |
| M      | Mitochondrion                               |
| MEFs   | Mouse embryonic fibroblasts                 |
| MDA    | Malondialdehyde                             |
| MSC    | Mesenchymal stem cell                       |
| MTC    | Masson's Trichrome                          |
| N      | Nucleus                                     |
| NIDDM  | Non-insulin dependent diabetes mellitus     |
| OD     | Optical density                             |
| PAS    | periodic acid-Schiff                        |
| PAX-4  | Paired box gene 4                           |
| PBS    | Phosphate buffer saline                     |
| PDX-1  | Pancreatic and duodenal homeobox 1          |

## ***LIST OF ABBREVIATIONS***

---

|        |                                        |
|--------|----------------------------------------|
| RER    | Rough endoplasmic reticulum            |
| SER    | Smooth endoplasmic reticulum           |
| SC     | Sinusoidal capillary                   |
| SG     | Secretory granule                      |
| SOD    | Super oxide dismutase                  |
| STZ    | Streptozotocin                         |
| T1DM   | Type 1 diabetes mellitus               |
| T2DM   | Type 2 diabetes mellitus               |
| TEM    | Transmission electron microscope       |
| V      | Vacuole                                |
| WJMSCs | Wharton's jelly mesenchymal stem cells |

## LIST OF FIGURES

| Figure No. | Title                                                                                                                                                                                   | Page |
|------------|-----------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------|------|
| Fig.1      | Mechanism of insulin action                                                                                                                                                             | 15   |
| Fig.2      | the structure of the insulin receptor                                                                                                                                                   | 15   |
| Fig.3      | Photomicrograph of spindle-shaped mesenchymal stem cells and rounded hematopoietic cells 3 days after culture in L-DMEM.                                                                | 59   |
| Fig.4      | Photomicrograph of homogeneous spindle-shaped mesenchymal stem cells after 8 days of culture showing bi- and tri-angled fibroblast-like cells.                                          | 59   |
| Fig.5      | Histogram showing the expression of surface markers specific to the mesenchymal stem cells.                                                                                             | 60   |
| Fig.6      | Flow cytometric analysis representing the relative cell number and fluorescence intensity, with unstained control cells and cells stained with antibodies against the surface proteins. | 61   |
| Fig.7      | Electron micrograph of spindle-shaped undifferentiated mesenchymal stem cells with a large nucleus and numerous cytoplasmic processes.                                                  | 63   |
| Fig.8      | Electron micrograph of an enlarged portion of undifferentiated mesenchymal stem cells showing rough endoplasmic reticulum, elongated mitochondria and a few lysosomes.                  | 63   |
| Fig.9      | Photomicrograph of mesenchymal stem cells after a week of differentiation in media containing 10 mmol/L nicotinamide, 1 mmol/L $\beta$ -mercaptoethanol.                                | 65   |
| Fig.10     | Photomicrograph of mesenchymal stem cells after five weeks of differentiation showing a large number of clusters.                                                                       | 65   |
| Fig.11     | Electron micrograph of differentiated mesenchymal stem cells showing a large number of secretory granules.                                                                              | 66   |
| Fig.12     | Electron micrograph of differentiated mesenchymal stem cells showing the insulin secretory granules each with a halo surrounding the granule.                                           | 66   |

|        |                                                                                                                                                                                                                                                                                                      |    |
|--------|------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------|----|
| Fig.13 | Gene expression of Insulin-1, Insulin-2, Pdx-1 and pax-4 in Insulin-producing cells shown in RT-PCR.                                                                                                                                                                                                 | 67 |
| Fig.14 | Photomicrograph of a section of the pancreas of control mice stained with hematoxylin-eosin showing the exocrine and endocrine portions (Islets of Langerhans).                                                                                                                                      | 75 |
| Fig.15 | Photomicrograph of a section of the pancreas of control mice stained with hematoxylin-eosin showing an Islet of Langerhans and exocrine acini.                                                                                                                                                       | 75 |
| Fig.16 | Photomicrograph of a section of the pancreas of control mice and immunostained using antibodies to insulin and counterstained with hematoxylin. The presence of insulin is indicated by strong cytoplasmic dark brown staining of $\beta$ -cells leaving the rest of the pancreatic tissue negative. | 76 |
| Fig.17 | Photomicrograph of a section of the pancreas of control mice and immunostained using antibodies to insulin. The presence of insulin is indicated by strong cytoplasmic dark brown staining of $\beta$ -cells leaving the rest of the pancreatic tissue negative.                                     | 76 |
| Fig.18 | Electron micrograph of a section of the pancreas of control mice showing the two types of pancreatic $\beta$ -cells; dark and light cells.                                                                                                                                                           | 77 |
| Fig.19 | Electron micrograph of a section of the pancreas of control mice showing the light cell. Note the large number of secretory granules and the rough endoplasmic reticulum.                                                                                                                            | 77 |
| Fig.20 | Enlarged portion of Fig. 19 showing a large number of secretory granules and the rough endoplasmic reticulum.                                                                                                                                                                                        | 78 |
| Fig.21 | Enlarged portion of Fig. 19 showing a large number of secretory granules and abundant mitochondria.                                                                                                                                                                                                  | 78 |
| Fig.22 | Electron micrograph of a section of the pancreas of control mice showing a dark cell with a large number of secretory granules with prominent halo and abundant mitochondria and Golgi apparatus.                                                                                                    | 79 |
| Fig.23 | Enlarged portion of Fig. 22 showing a large number of secretory granules with a prominent                                                                                                                                                                                                            | 79 |

|        |                                                                                                                                                                                                              |    |
|--------|--------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------|----|
|        | halo. Note the abundant mitochondria and Golgi apparatus.                                                                                                                                                    |    |
| Fig.24 | Photomicrograph of a section of the pancreas of diabetic mice stained with hematoxylin-eosin showing congestion of blood vessels and destructed islets.                                                      | 80 |
| Fig.25 | Photomicrograph of a section of the pancreas of diabetic mice stained with hematoxylin-eosin showing necrosis and vacuolation of cells of the islet of Langerhans.                                           | 80 |
| Fig.26 | Photomicrograph of a section of the pancreas of diabetic mice stained with hematoxylin-eosin showing congestion of blood vessels and infiltration of lymphocytes into the blood.                             | 81 |
| Fig.27 | Photomicrograph of a section of the pancreas of diabetic mice immune-stained using antibodies to insulin showing reduced insulin immune-reactivity represented by minimal dark brown insulin staining.       | 82 |
| Fig.28 | Photomicrograph of a section of the pancreas of diabetic mice immune-stained using antibodies to insulin showing necrosis and vacuolation of cells of the Islet of Langerhans with reduced insulin staining. | 82 |
| Fig.29 | Electron micrograph of a section of the pancreas of diabetic mice showing shrinkage of the two types of pancreatic $\beta$ -cells.                                                                           | 83 |
| Fig.30 | Electron micrograph of the pancreas of diabetic mice showing a light cell showing pyknotic nucleus, vacuoles in the cytoplasm and lack of the secretory granules.                                            | 83 |
| Fig.31 | Enlarged portion of Fig. 30 showing vacuoles in the cytoplasm and mitochondria.                                                                                                                              | 84 |
| Fig.32 | Enlarged portion of Fig. 30 showing fragmentation of rough endoplasmic reticulum and vacuoles in the cytoplasm.                                                                                              | 84 |
| Fig.33 | Electron micrograph of the pancreas of diabetic mice showing a dark cell with an indented nucleus, vacuoles in the cytoplasm and lack of the secretory granules.                                             | 85 |
| Fig.34 | Electron micrograph of the pancreas of diabetic                                                                                                                                                              | 85 |

|        |                                                                                                                                                                                                                                                             |    |
|--------|-------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------|----|
|        | mice showing a dark cell lacks of organelles and granules.                                                                                                                                                                                                  |    |
| Fig.35 | Photomicrograph of a section of the pancreas of diabetic mice treated with undifferentiated stem cells stained with hematoxylin-eosin showing the exocrine and endocrine portions. Note the congestion of blood vessels with infiltration of lymphocytes.   | 86 |
| Fig.36 | Photomicrograph of a section of the pancreas of diabetic mice treated with undifferentiated stem cells stained with hematoxylin-eosin showing an islet of Langerhans with several vacuoles.                                                                 | 86 |
| Fig.37 | Photomicrograph of a section of the pancreas of diabetic mice treated with undifferentiated stem cells immunostained using antibodies to insulin showing insulin immune-reactivity represented by dark brown insulin staining.                              | 87 |
| Fig.38 | Photomicrograph of a section of the pancreas of diabetic mice treated with undifferentiated stem cells and immunostained using antibodies to insulin showing amelioration of insulin immune-reactivity represented by moderate dark brown insulin staining. | 87 |
| Fig.39 | Electron micrograph of a section of the pancreas of diabetic mice treated with undifferentiated stem cells showing a light cell with several secretory granules.                                                                                            | 88 |
| Fig.40 | Enlarged portion of Fig. 39 showing the secretory granules and rough endoplasmic reticulum of the light cell.                                                                                                                                               | 88 |
| Fig.41 | Electron micrograph of a section of the pancreas of diabetic mice treated with undifferentiated stem cells showing a dark cell with normal secretory granules.                                                                                              | 89 |
| Fig.42 | Enlarged portion of Fig. 41 showing the secretory granules of the dark cell.                                                                                                                                                                                | 89 |
| Fig.43 | Photomicrograph of a section of the pancreas of diabetic mice treated with insulin-producing cells stained with hematoxylin-eosin showing normal islet of Langerhans.                                                                                       | 90 |
| Fig.44 | Photomicrograph of a section of the pancreas of                                                                                                                                                                                                             | 90 |

|        |                                                                                                                                                                                                                   |     |
|--------|-------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------|-----|
|        | diabetic mice treated with insulin-producing cells and immunostained using antibodies to insulin showing amelioration of insulin immune-reactivity represented by intense dark brown insulin staining.            |     |
| Fig.45 | Electron micrograph of a section of the pancreas of diabetic mice treated with insulin-producing cells showing a normal light cell with numerous secretory granules.                                              | 91  |
| Fig.46 | Electron micrograph of a section of the pancreas of diabetic mice treated with insulin-producing cells showing the normal structure of rough endoplasmic reticulum, mitochondria and abundant secretory granules. | 91  |
| Fig.47 | Electron micrograph of a section of the pancreas of diabetic mice treated with insulin-producing cells showing a normal dark cell.                                                                                | 92  |
| Fig.48 | Electron micrograph of a section of the pancreas of diabetic mice treated with insulin-producing cells showing healthy abundant secretory granules, mitochondria and Golgi apparatus.                             | 92  |
| Fig.49 | Photomicrograph of a section of the liver of control mice stained with hematoxylin-eosin showing the normal organization of liver parenchyma.                                                                     | 100 |
| Fig.50 | Photomicrograph of a section of the liver of control mice stained with hematoxylin-eosin showing cords of hepatocytes separated by sinusoidal capillaries.                                                        | 100 |
| Fig.51 | Photomicrograph of a section of the liver of control mice stained with hematoxylin-eosin showing a central vein surrounded by hepatic strands.                                                                    | 101 |
| Fig.52 | Photomicrograph of a section of the liver of control mice stained with hematoxylin-eosin showing hepatocytes and blood sinusoids lined by endothelial cells and macrophages.                                      | 101 |
| Fig.53 | Photomicrograph of a section of the liver of control mice stained with periodic acid-Schiff showing the normal organization of glycogen granules within the hepatocytes.                                          | 102 |