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XENOTRANSPLANTATION OF CAPRINE PANCREATIC ISLETS IN DIABETIC MICE

HOMAYOUN HANI

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By

HOMAYOUN HANI

Thesis Submitted to the School of Graduate Studies, Universiti Putra Malaysia, in Fulfillment of the Requirements for the Degree of Doctor of Philosophy

December 2013

DEDICATION

I dedicate with appreciation to my parents, Javad and Maryam
to my brothers, Reza and Hadi
to my beloved wife, Kazhal Sarsaifi,
for their great support, patience, and motivation

Abstract of thesis presented to the Senate of Universiti Putra Malaysia in fulfillment of the requirement for the degree of Doctor of Philosophy

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Chairman : Zeenathul Nazariah Allaudin, PhD
Faculty : Institute of Bioscience

Diabetes is one of the aggressive metabolic syndromes, which allied with high levels of blood glucose subsequent of imperfections in insulin production that causes glucose to increase in the body. For decades, excellent improvement has been obtained in clinical transplantation of pancreatic islet. Because of that, islet transplantation has become the main alternative cure for type 1 diabetics. However, important obstacles still remain to use of islet transplants routinely as a treatment choice. The deficiency of human islet donors makes the search for alternative islet sources mandatory for future developments in pancreatic islet transplantation. Venturing into xenotransplantation provides tremendous extensive sources of islet cells. The improvement of genetically engineered pigs expressing human complement regulatory proteins to defeat immune damaging pathways has been promising; however, the necessity of massive immunosuppressive and the concern for zoonotic viral transmission are some of the constraints. The present investigation unravels the potential of caprine islets as an alternative islet source for xenotransplantation. One of the main reasons for performing this preliminary study was that religious and cultural factors in countries including Malaysia may favor goats over pigs as xenograft donors. However, potential limitations associated with the use of caprine donors must be recognized. First, the mean yield of 120,000 islets per adult goat is somewhat lower than the reported yield of up to 360,000 islets obtained from adult pigs. Second, the efficacy of goat insulin in humans is not known. The amino acid sequence of goat insulin differs from that of human insulin at four residues, compared to pig insulin which differs at only one. However, bovine insulin, which differs at three of the same four residues as goat insulin, has been shown to control diabetes in humans. Furthermore, should there be a problem with goat insulin, it would be feasible to genetically modify goats to produce human insulin. This study aimed i) to characterize viable caprine islets, ii) to inspect the impact of antioxidant and secretagogues factors *in vitro* maintenance of caprine islets and their viability in culture media iii) to scrutinize the *in vitro* enhancing factors for insulin promoter gene and protein expression in caprine islets, iv) to assess the functionality of grafted caprine islets in immunized diabetic murine models.

Microscopic characterization of viable caprine islets was carried out to evaluate endocrine cell types. Caprine islets were successfully obtained using a collagenase-

based digestion, isolation and Euro-Ficoll density gradient purification technique at optimum pH (= 7.4). Purity and viability of islets were determined by dithizone and FDA/PI staining respectively. The viability of purified islet cells exceeded 90%. Caprine islet morphological assessment and cyto-architectural study were carried out using single and multiple immunostaining for insulin, glucagon and somatostatin and then assessed by confocal microscopy and flow cytometry. Under the confocal microscope, the mean percentage of β-cell, α-cell and δ-cell in different layers of purified islet were 38.01 ± 12.47%, 30.33 ± 12.33 and 2.15 ± 1.17%, respectively. Majority of β-cells were centralized whilst the other two cell types were placed in the peripheral regions. A similar pattern of abundance of β-cell, α-cell and δ-cell population was determined by flow cytometry analysis (37.52 ± 9.74%, 31.72 ± 5.67%, and 2.73 ± 2.73% respectively). Flow cytometry findings of the endocrine cell population within caprine islets were consistent with the microscopic investigation results. The morphological study of caprine islet revealed arrangement of the different islet cell types, which can lead to better understanding of different cell type interactions in caprine islets. The cytoarchitectural study of caprine islet can be occasioned to the comparison of similarity and dissimilarity of caprine islet cytoarchitectural features with other species islets and their physiological structures. However, anatomical study of caprine islet was conducted to *in vitro* and *in vivo* function assessment.

Two agents of antioxidant and secretagogue were considered to enhance viability and functionality of caprine islets. Tocopherol as an antioxidant agent could offer anti-apoptosis reaction and secretagogue agent, 3-isobutyl-1-methylxanthine (IBMX), enhance insulin secretion of caprine islets in the culture. The impact of supplementing antioxidant (tocopherol) and secretagogue factors (IBMX) on caprine islet viability during a short period maintenance of caprine islet culture was also assessed. The treated caprine islets with mentioned agents showed stability of islet morphology in cell culture, viability and functionality due to microscopy observation, FDA/PI staining and insulin secretion while stimulated by a high and low glucose stimulation, respectively (Day 1; 0.24 ± 0.09 µg/L, 0.13 ± 0.02 µg/L, Day 3; 0.19 ± 0.07 µg/L, 0.07 ± 0.02 µg/L, Day 5; 0.22 ± 0.05 µg/L, 0.11 ± 0.02 µg/L). These results can be promoted via molecular and gene expression studies on caprine islet.

The insulin promoter gene (PDX1) as one of the most important genes in pancreas and islet was studied in caprine islets. Because it plays essential roles in pancreas and islet expansion, pancreas growth, islet formation within the pancreas and insulin secretion from β-cells. The PDX-1 and its protein expression were simultaneously assessed in the supplemented caprine islet culture. The results showed PDX1 gene up-regulation during five days of tocopherol and 3-isobutyl-1-methylxanthine supplemented caprine islet culture compared with the control group, serum-free media, with the relative quantification (RQ) value, day 1; 7.85 ± 1.20, day 3; 1.84 ± 0.14, and day 5; 6.80 ± 2.08 fold. Enhancement of PDX-1 expression in caprine islet results to produce more effective islets for *in vivo* study and xenotransplantation approach.

The final aim of this study was to assess caprine islet functionality after xenotransplantation into a diabetic murine model. The optimal islets (size range

between 50-250 µm) of viable purified caprine islets were transplanted into the recipient group (immunosuppressed diabetic mice) and compared with control groups of non-diabetic mice, un-grafted diabetic mice without immunosupresive drug injection, immunosuppressed mice with sham graft and islets grafted mice without immunosuppressive drug injection. Glucose tolerance test, blood glucose monitoring and microscopic examination of transplanted graft collectively indicated a reversion of diabetic status in STZ induced immunosuppressed mice. Non-fasting blood glucose level, 8.04 ± 0.44 mM/L, decreased from 23.3 ± 5.4 mM/L, meanwhile serum insulin level increase from 0.01 ± 0.001 µg/L to 0.56 ± 0.17 µg/ L and recipient mice body weights increased from 23.64 ± 0.31g to 25.85 ± 0.34 g ($p < 0.05$).

In conclusion, the combination of tocopherol and IBMX was capable to impair the rate of apoptosis, improve the viability of caprine islets for short period culture and enhance the duodenal homeobox gene and protein expression. It might be considered a potential treatment to improve islet viability *in vitro* before islet transplantation. As the first attempt of using purified caprine islets, results indicate that the grafted islets were capable to retrieval diabetes in immunosuppressed STZ-injected mice.

Abstrak tesis yang dikemukakan kepada Senat Universiti Putra Malaysia sebagai memenuhi keperluan untuk ijazah Doktor Falsafah

XENOTRANSPLANTASI PEPULAU PANKREAS KAPRIN DI DALAM TIKUS DIABETES

Oleh

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Diabetes merupakan satu daripada sindrom metabolisma agresif yang terkait dengan aras glukosa darah tinggi akibat daripada kekurangan penghasilan insulin yang menyebabkan peningkatan glukosa dalam badan. Sejak beberapa dekad kebelakangan ini peningkatan yang cemerlang telah berlaku dalam transplantasi pepulau klinikal. Oleh sebab itu, transplantasi sel pepulau telah menjadi penyembuh alternatif utama untuk diabetes jenis 1. Walaubagaimanapun terdapat beberapa halangan besar dalam penggunaan rutin transplantasi pepulau sebagai rawatan pilihan. Kekurangan penderma pepulau manusia menyebabkan pencarian sumber pepulau alternatif perlu dilakukan untuk perkembangan masa hadapan dalam transplantasi pepulau pankreas. Penerokaan xenotransplantasi telah membekalkan sumber pepulau yang tiada had. Perkembangan babi transgenik yang menekspresikan protein pengawalaturan pelengkap untuk mengatasi imun pemusnah laluan-laluan adalah menggalakkan; walau bagaimanapun, terdapat beberapa kekangan termasuk imunotindasan teruk dan kerisauan terhadap kemungkinan berlakuya pemindahan virus zoonosis. Kajian ini menguraikan potensi pepulau kaprin sebagai sumber pepulau alternatif untuk xenotransplantasi. Salah satu sebab utama untuk melaksanakan kajian awal ini adalah kerana faktor-faktor agama dan budaya di negara seperti Malaysia yang memilih kambing lebih daripada babi sebagai penderma xenograft. Walau bagaimanapun, had yang berkait dengan penggunaan penderma kaprin haruslah dikenalpasti. Pertama, min hasil 120,000 pulau kecil setiap kambing dewasa adalah lebih rendah daripada hasil yang dilaporkan sehingga 360,000 pulau kecil yang diperolehi daripada babi dewasa. Kedua, keberkesanan insulin kambing pada manusia tidak diketahui. Urutan asid amino insulin kambing adalah berbeza dengan insulin manusia di empat residu berbanding dengan insulin babi yang berbeza hanya satu. Walau bagaimanapun, insulin bovin, yang berbeza di tiga daripada empat residu yang sama pada insulin kambing, telah terbukti dapat mengawal diabetes pada manusia. Tambahan pula, jika terdapat masalah dengan insulin kambing, ia boleh diatasi dengan mengubah suai genetik kambing untuk menghasilkan insulin manusia. Tujuan kajian ini ialah i) untuk menciri pepulau kaprin hidup, ii) untuk mengkaji impak penyenggaraan faktor-faktor antioksidan dan sekretagog *in vitro* pepulau kaprin dan kebolehhidupannya dalam medium kultur, iii) untuk mengkaji faktor perangsang *in vitro* gen penggalak insulin dan protein ekspresi dalam pepulau kaprin dan iv) untuk menilai kefungsian sel pepulau kaprin yang dicantum dalam model murin diabetes terimun.

Pencirian mikroskopi pepulau kaprin hidup telah dijalankan untuk menilai jenis-jenis sel endokrin. Pepulau kaprin telah berjaya diperolehi mengguna pencernaan berasaskan kolagenase, pemencilan dan teknik penulenan cerun ketumpatan Euro-Ficoll pada pH optimum (7.4). Ketulenan sel pepulau dan kebolehhidupannya telah ditentukan menggunakan perwarnaan ditizon dan FDA/PI. Kebolehhidupan sel pepulau tertulen melebihi 90%. Penilaian morfologi pepulau kaprin dan kajian reka bentuk sito telah dijalankan menggunakan imunopewarnaan tunggal dan berbilang untuk insulin, glukagon dan somatostatin dan kemudian dinilai melalui mikroskopi konfokal dan sitometri aliran. Daripada mikroskopi konfokal, min peratusan β-sel, α-sel dan δ-sel dalam pelbagai lapisan pepulau tertulen masing-masing adalah 38.01 ± 12.47 30.33 ± 12.33 and 2.15 ± 1.17%. Kebanyakan sel β-sel terpusat manakala dua jenis sel lagi terletak di kawasan pinggiran. Pola populasi β-sel, α-sel dan δ-sel berlebihan yang sama telah ditentukan melalui analisis sitometri aliran (masing-masing 37.52 ± 9.74, 31.72 ± 5.67, and 2.73 ± 2.73). Sitometri aliran membuktikan ketekalan hasil penyiasatan mikroskopi yang dijalankan terhadap populasi sel endokrin kaprin. Kajian morfologi pulau kecil kambing menunjukkan perbezaan pada susunan sel pulau kecil yang boleh membawa kepada pemahaman yang lebih baik mengenai interaksi pelbagai jenis sel yang berbeza dalam pulau kecil kambing. Kajian reka bentuk sito pulau kecil kambing boleh disebabkan kepada perbandingan persamaan dan perbezaan ciri-ciri kajian reka bentuk sito pulau kecil kambing dengan struktur fisiologikal mereka. Walau bagaimanapun, kajian anatomi pulau kecil kambing telah dijalankan untuk penilaian fungsi *in vitro* dan *in vivo*.

Dua agen antioksidan dan "secretagogue" dianggap untuk meningkatkan daya maju dan fungsi pulau kecil kambing. Tokoferol sebagai agen antioksidan boleh menawarkan tindak balas anti-apoptosis dan agen secretagogue, 3-isobutyl-1-methylxanthine (IBMX), meningkatkan rembesan insulin pulau kecil kambing dalam kultur. Impak penambahan faktor antioksidan (tokoferol) dan sekretagog (3-isobutil-1-metilxantin) terhadap kebolehhidupan pepulau kaprin dalam penyelenggaran jangka pendek kultur sel pepulau kaprin juga telah dinilaikan. Pepulau kaprin yang telah dirawat dengan agen-agen tersebut menunjukkan kestabilan morfologi pepulau dalam kultur sel, kebolehhidupan dan kefungsian melalui pencerapan mikroskopi, pewarnaan FDA/PI dan rembesan insulin apabila dirangsang dengan kepekatan glukosa rendah dan tinggi (masing-masing, Hari 1; 0.24 ± 0.09 µg/L, 0.13 ± 0.02 µg/L, Hari 3; 0.19 ± 0.07 µg/L, 0.07 ± 0.02 µg/L, Hari 5; 0.22 ± 0.05 µg/L, 0.11 ± 0.02 µg/L). Keputusan ini boleh disokong melalui kajian molekul dan ekspresi gen pulau kecil kambing.

Promoter gen insulin (PDX1) sebagai salah satu gen yang paling penting dalam pankreas dan pulau kecil dikaji dalam pepulau kecil kambing. Oleh kerana ia memainkan peranan penting dalam pankreas dan pengembangan pulau kecil, pertumbuhan pankreas, pembentukan pulau kecil dalam pankreas dan insulin dari β-sel. PDX1 dan ekspresi proteinnya telah dinilaikan serentak dalam kultur sel pepulau kaprin yang menerima penambah. Hasil penilaian ini menunjukkan pengawalaturan gen PDX1 menaik dalam tempoh lima hari pengkulturan pepulau kaprin tertambah tokoferol dan 3-isobutil-1-metilxantin berbanding kumpulan kawalan dalam medium bebas serum, dengan nilai pengkuantitian bandingan (RQ), hari 1; 7.85 ± 1.20, hari 3; 1.84 ± 0.14,

and hari 5; 6.80 ± 2.08 kali ganda. Kajian *in vivo* dan pendekatan xenotransplantasi menunjukkan peningkatan dalam ekspresi gen PDX-1 di pulau kecil kambing yang menunjukkan penghasilan pulau kecil yang lebih efektif. Objektif terakhir dalam kajian ini ialah untuk menilai kefungsian pepulau kaprin selepas xenotransplantasi dalam model murin diabetes. Pepulau optimum (julat saiz antara 50 dan 250 µm) pepulau kaprin tulen hidup telah ditransplantasikan dalam kumpulan penerima (tikus diabetes terimunotindas) dan dibanding dengan tikus bukan diabetes kumpulan kawalan, tikus diabetes tidak ditransplantasi tanpa suntikan dadah imunotindas, tikus terimuntindas dengan transplantasi palsu dan tikus yang ditransplantasi dengan pepulau tanpa suntikan dadah imunotindas. Ujian tolerans glukosa, pemantauan glukosa darah dan pemeriksaan mikroskopi cantuman yang ditransplantasi pada keseluruhannya menunjukkan pembalikan kepada status diabetes dalam tikus diimuntindas dengan STZ. Aras glukosa darah ketika tidak berpuasa, menurun daripada 23.3 ± 5.4 mM/L kepada 8.04 ± 0.44 mM/L, manakala aras insulin serum meningkat daripada 0.01 ± 0.001 µg/L kepada 0.56 ± 0.17 µg/L dan berat badan tikus penerima meningkat daripada 23.64 ± 0.31g kepada 25.85 ± 0.34 g ($p < 0.05$).

Kesimpulannya, gabungan tokoferol dan IBMX dapat mengurangkan kadar apoptosis, meningkatkan kebolehhidupan pepulau kaprin dalam kultur jangka pendek dan meningkatkan gen homeobox duodenum dan ekspresi protin. Ia mungkin boleh dipertimbangkan sebagai rawatan yang berpotensi untuk memperbaiki kebolehhidupan pepulau *in vitro* sebelum transplantasi pepulau. Sebagai usaha pertama dalam mengunakan pepulau kaprin tertulen, hasil kajian menunjukkan pepulau yang digraft berupaya untuk memulihkan diabetes dalam tikus terimuntindas yang telah disuntik dengan STZ.

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I certify that a Thesis Examination Committee has met on the Xth of x 2013 to conduct the final examination of Homayoun Hani on his thesis entitled “Xenotransplantation of Caprine Pancreatic Islets in Diabetic Mice" in accordance with the universities and university colleges Act 1971 and the Constitution of the Universiti Putra Malaysia [P.U. (A) 106] 15 March 1998. The Committee recommends that the student be awarded the Doctor of Philosophy.

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TABLE OF CONTENTS

		Page
ABSTRACT		iii
ABSTRAK		vi
ACKNOWLEDGEMENTS		ix
APPROVAL		x
DECLARATION BY GRADUATE STUDENT		xii
DECLARATION BY MEMBERS OF SUPERVISORY COMMITTEE		xiii
LIST OF FIGURES		xix
LIST OF TABLES		xxii
LIST OF ABBREVIATIONS		xxiii
CHAPTER		
1	**INTRODUCTION**	**1**
	1.1 Background	1
	1.2 Problem Statements	3
	1.3 Objectives	3
	1.4 Null Hypothesis	3
2	**LITERATURE REVIEW**	**4**
	2.1 Diabetes Prevalence	4
	2.2 Pancreas	7
	2.3 Pancreatic Islets of Langerhans	8
	2.3.1 Islets cell types	10
	2.3.1.1 α-cells	10
	2.3.1.2 β-cells	10
	2.3.1.3 δ-Cells	10
	2.3.1.4 PP Cells	11
	2.3.1.5 ε-Cells	11
	2.3.2 Islets of Langerhans in humans	11
	2.3.3 Islets of Langerhans in porcine	12
	2.3.4 Islets of Langerhans in rodents	12
	2.3.5 Islets of Langerhans in piscine	13
	2.3.6 Islets of Langerhans in ruminants	14
	2.3.6.1 Bovine Islets	14
	2.3.6.2 Caprine Islets	14
	2.3.6.3 Ovine Islets	14
	2.4 Islet Culture	14
	2.5 Diabetes	15
	2.5.1 Type 1 diabetes cures	16
	2.5.1.1 Prevention	17
	2.5.1.2 Transplantation	17
	2.5.1.3 Xenotransplantation	19

2.6 Islet Topography 23
2.6.1 Confocal Microscopic Study on Islets of Langerhans 23
2.7 Flowcytometry for islets of Langerhans 24
2.8 Real-Time PCR for insulin promoter gene 24
2.8.1 Insulin Promotor Gene (pancreatic and duodenal homeobox 1; PDX1) 25

3 CYTOARCHITECTURAL AND MORPHOLOGICAL STUDY OF CAPRINE ISLETS 27
3.1 Introduction 27
3.2 Materials and Methods 28
3.2.1 Caprine Pancreas Sample Collection 28
3.2.2 Isolation of Islets of Langerhans 28
3.2.3 Islet Purification 28
3.2.4 Islet Viability Assessment 29
3.2.4.1 Trypan Blue Staining 29
3.2.4.2 Dithizone Staining 29
3.2.4.3 Indirect Immunoperoxidase Test 30
3.2.4.4 Apoptosis Detection by FDA/PI Viability Staining 30
3.2.5 Islet Cell Culture System 30
3.2.6 Immunofluorescence / Confocal Microscopy 30
3.2.7 Islets Immuno-fluorescent staining for Flowcytometry Assessment 31
3.2.8 Statistical Analysis 31
3.3 Results 31
3.3.1 Isolation Procedure 31
3.3.2 Islet Purification 32
3.3.3 Viability Assessment 33
3.3.3.1 Trypan blue staining 33
3.3.3.2 FDA/PI staining 33
3.3.4 Indirect immunoperoxidase staining on purified Caprine islets 34
3.3.5 Islet Cell Culture 35
3.3.6 Assessment of Caprine Islets by Confocal Microscopy 35
3.4 Discussion and Conclusion 46

4 IMPROVEMENT IN CULTURE AND MAINTENANCE OF CAPRINE ISLETS 50
4.1 Introduction 50
4.2 Materials and Methods 51
4.2.1 Experimental Design and Islet Cell Culture preparation 51
4.2.2 Serum Free Media 51

4.2.3 Serum Added Media 51
4.2.4 Islet Culture in RPMI-1640 Plus 3-Isobutyl-1-Methyl Xanthin (IBMX) 52
4.2.5 Caprine islet maintenance in RPMI-1640 with tocopherol (α- and γ-tocopherol) 52
4.2.6 IBMX and Tocopherol 52
4.2.7 Glucose-Stimulated Insulin Secretion (GSIS) 52
4.2.8 Viability Assessment of Islets 53
4.2.9 Statistical Analysis 53
4.3 Results 53
4.3.1 Islet Morphology Assessment 53
4.3.2 Viability Determination 55
4.3.3 Insulin Secretion Assessment 57
4.4 Discussion and Conclusion 59

5 PROTECTION OF CULTURED CAPRINE ISLETS IN ANTIOXIDANT SUPPLEMENTED MEDIA VIA PDX1 REGULATION 63
5.1 Introduction 63
5.2 Materials and Methods 65
5.2.1 Maintenance of Caprine Islets in Different Supplemented Media 65
5.2.2 Primers for Gene Expression of PDX-1 65
5.2.3 RNA Extraction 65
5.2.4 Reverse Transcriptase PCR 66
5.2.5 Agarose Gel Purification 66
5.2.6 Quantitative Reverse Transcriptase Real-time PCR 67
5.2.7 Reverse Transcriptase Real-time PCR Efficiency 67
5.2.8 Analysis of Gene Expression 67
5.2.9 Quantitative Levels and Comparative CT ($2^{-\Delta\Delta CT}$) Method 68
5.2.10 SDS-PAGE Electrophoresis 68
5.2.11 Western Blotting for PDX1 69
5.2.12 Statistical Analysis 69
5.3 Results 69
5.3.1 Purified Islet Viability Assessment 69
5.3.2 Primer Specificity Determination 69
5.3.3 Real-time PCR Efficiency Linearity 71
5.3.4 Relative Quantification Method 74
5.3.5 PDX-1Gene Expression in Treated and Untreated Islets 84
5.3.6 Protein Size Estimation by SDS-PAGE 85
5.4 Discussion and Conclusion 86

6 TRANSPLANTATION OF CAPRINE ISLETS OF LANGERHANS INTO DIABETIC MURINE MODEL 90
6.1 Introduction 90
6.2 Materials and Methods 91
6.2.1 Sample Collection 91
6.2.2 Processing of Caprine Islets 91
6.2.3 Dithizone Staining 91
6.2.4 Apoptosis detection in pancreatic islets 91
6.2.5 Insulin Secretion Assessment by Using ELISA 91
6.2.6 Diabetes Induction in Mice Using STZ 92
6.2.7 Blood and Urine Glucose Determination in Diabetic Mice 92
6.2.8 Diabetic Mice Maintenance 92
6.2.9 Immunosuppressive Drug Treatment in Mice prior of Islets Xenograft Procedures 93
6.2.10 Mice Anaesthesia and Preparation for Islet Xenograft 93
6.2.11 Islet Preparation for Xenotransplantation 93
6.2.12 Islet Xenotransplantation Surgical Procedures (Transplant islets to sub-capsular kidney pouch) 93
6.2.13 Monitoring Mice Recovery 94
6.2.14 Islet Graft Monitoring 94
6.2.15 Intraperitoneal Glucose Tolerance Test (IPGTT) 94
6.2.16 Histological Procedures on Islets Graft 95
6.2.17 Statistical Analysis 95
6.3 Results 95
6.3.1 Islet Isolation and Purification 95
6.3.2 Islet viability assessment 96
6.3.3 Insulin Secretion Assessment 96
6.3.4 Diabetes Induction Assessment 97
6.3.5 Urine Glucose Determination in Diabetic Mice 97
6.3.6 Grafted Islet Insulin Secretion Assessment 98
6.3.7 Blood Glucose and Body Weight Monitoring During the Experimental Period and Evaluation of Caprine Islet Quality 99
6.3.8 Intraperitoneal Glucose Tolerance Test (IPGTT) 101
6.3.9 Islet Xeno-graft Histology Characterization 103
6.4 Discussion and Conclusion 104

7 GENERAL DISCUSSION, CONCLUSION AND RECOMMENDATIONS FOR FUTURE RESEARCH 106
7.1 General Discussion 106
7.2 Conclusion 108
7.3 Recommendations for Future Research 108

REFERENCES 110
APPENDICES 140
BIODATA OF STUDENT 152
LIST OF PUBLICATIONS 153

LIST OF FIGURES

Figure		Page
2.1	Global diabetes prevalence	5
2.2	Type 1 diabetes development by antigen-presentation incidents	7
2.3	Structure of the pancreas	8
2.4	Schematic arrangement of endocrine cells and blood vessels in islet of Langerhans	9
2.5	The molecules involved during pancreatic development	25
3.1	DTZ staining of the caprine islets after isolation	32
3.2	DTZ staining of the caprine islets after purification	33
3.3	Trypan blue staining of the caprine islets after isolation	33
3.4	Determination of cell viability in fresh caprine pancreatic islets	34
3.5	Immunoperoxidase staining of purified islet clusters	35
3.6	Short-term caprine pancreatic islet culture in RPMI-1640	35
3.7	Immunofluorescent staining of caprine islet different cell types for confocal microscopy observation	36
3.8	Immunostaining of insulin secreting cells in isolated caprine pancreatic islet	37
3.9	Optical sectioning of caprine islets after immunostaining of insulin secreting cells	37
3.10	Immunostaining of glucagon secreting cells in isolated caprine pancreatic islet	38
3.11	Optical sectioning of caprine islets after immunostaining of glucagon secreting cells	38
3.12	Immunostaining of somatostatin secreting cells in isolated caprine pancreatic islet	38
3.13	Optical sectioning of caprine islets after immunostaining of somatostatin secreting cells	39

3.14	Three-dimensional analysis of caprine pancreatic islet	39
3.15	Immunofluorescent staining of large islet different cell types for confocal microscopy observation	40
3.16	Insulin secreting cells immunofluorescent staining of different sizes of isolated caprine islets	41
3.17	Glucagon secreting cells immunofluorescent staining of different sizes of isolated caprine islets	41
3.18	Somatostatin secreting cells immunofluorescent staining of different sizes of isolated caprine islets	42
3.19	Analysis of islet size distribution and cellular composition among caprine islets	42
3.20	Caprine islets single cell flow cytometry assessment	44
3.21	Estimate of islet cell populations using confocal microscopy and flow cytometry	45
4.1	Short-term caprine islet culture in different supplemented media	54
4.2	Caprine pancreatic islets viability assessment by FDA/PI staining	55
4.3	GSIS assessment of isolated caprine islets in different treatments	58
4.4	GSIS test on isolated caprine pancreatic islets	59
5.1	Identification of the PDX1, β-Actin and GAPDH gene primer sets specificity	70
5.2	Gel electrophoresis of gradient PCR for PDX1 gene primer sets	71
5.3	Standard curve of β-actin gene	72
5.4	Standard curve of GAPDH gene	73
5.5	Standard curve of PDX1 gene	74
5.6A	Gel electrophoresis of RT real-time PCR PDX1 and reference gene products after one day	76
5.6B	Gel electrophoresis of RT real-time PCR PDX1 and reference gene products after three days	78
5.6C	Gel electrophoresis of RT real time PCR PDX1 and reference gene products after five days	80
5.7A	Graph of the expression ratio for caprine islet PDX1 gene, while	81

	exposed to treatments in culture at day 1	
5.7B	Graph of the expression ratio for caprine islet PDX1 gene, while exposed to treatments in culture at day 3	82
5.7C	Graph of the expression ratio for caprine islet PDX1 gene, while exposed to treatments in culture at day 5	83
5.8	SDS-PAGE of caprine islets total protein	85
5.9	Western blot analysis for PDX1 protein expression in caprine islet cells	86
6.1	Caprine islet transplant to sub-capsular kidney pouch	94
6.2	DTZ stained caprine pancreatic islets under inverted microscope	96
6.3	Cell viability assessment	96
6.4	In vitro insulin release from caprine islets in response to glucose challenge	97
6.5	Urine glucose level check using Accu-Chek Diabura-Test 500 strips	98
6.6	Insulin secretion of diabetic mice	98
6.7	Caprine islets graft effect on glycemic control in diabetic mice	99
6.8	Blood glucose levels of experimental groups	100
6.9	Mice body weight monitoring during the experimental period	101
6.10	Changes in body weights of the mice after STZ, sham thalidomide and islets transplantation treatment	101
6.11	Intra-peritoneal glucose tolerance tests were performed in fasted mice at week two post-transplantation of caprine islets	102
6.12	Statistical analysis of IPGTT data of GDT, SDT, DC, and NC experimental groups at week two post-transplantation of caprine islets.	103
6.13	H&E and Immunohistological staining of caprine islet grafts from BALB/c mice at the end of the experiment.	104

LIST OF TABLES

Table		Page
2.1	Autoantibodies against pancreatic antigens in type 1 diabetes	6
3.1	Caprine islet composition	46
4.1	Percentage of caprine islet viability in serum-free, 5% serum, IBMX, tocopherol and combination of IBMX/tocopherol supplemented media during five days after isolation	56
4.2	Comparison of insulin secretion assessment of caprine islets in serum-free media, 5% serum, IBMX, tocopherol and combination of IBMX/tocopherol	58
5.1	Statistical analysis showing significant differences between the PDX1 gene RQ value of islets treated with serum-free, 5% serum, IBMX, tocopherol and combination of IBMX/tocopherol supplemented media at day 1, day3 and day5.	84
5.2	Statistical analysis showing significant differences between PDX1 gene RQ value of different days for each treatment	84
6.1	Experimental mice biodata	97

LIST OF ABBREVIATIONS

3D	Three Dimensional
µl	Microliter
µm	Micrometer
µM	Micromolar
2D	Two Dimensional
Ab	Antibody
ABTS	2,2'-Azino-Di (3-Ethyl) Benzthiazoline Sulphonic Acid
ANOVA	Analysis of Variance
ATP	Adenosine Triphosphate
ATV	Antibiotic Trypsin Versene
BBs	Brockmann Bodies
BCG	Bacillus Calmette Guerin
BD	Becton Dickinson
BG	Blood Glucose
bp	Base Pair
BSA	Bovine Serum Albumin
C	Centigrade
Ca	Calcium
CAM	Cell Adhesion Molecule
cDNA	Complementary Deoxyribonucleic Acid
CDX2	Caudal Type Homeobox 2
cm	Centimeter
cm^2	Square Centimeter
CMRL 1066	Connaught Medical Research Laboratories 1066
CRD	Completely Randomized Design
CT	Threshold Cycle
DAB	3,3-Diaminobenzidine
DCCT	Diabetes Control and Complications Trial
dl	Deciliter

DMSO	Dimethylsulfoxide
DNA	Deoxyribonucleic Acid
DNase	Deoxyribonuclease
dNTP	Deoxyribonucleotide Triphosphate
DPX	Dibutyl Phthalate Xylene
DTZ	Dithizone
E	Efficiency
EDTA	Ethylenediaminetetraacetic
ELISA	Enzyme-Linked Immunosorbent Assay
em	Emission
FBS	Fetal Bovine Serum
F-cells	Polypeptide Producing Cells
FCS	Fetal Calf Serum
FDA	Fluorescein Diacetate
fg	Femtogram
FITC	Fluorescein Isothiocyanate
G	Gram
GAD	Glutamic Acid Decarboxylase
GAD65	Glutamic Acid Decarboxylase
GAPDH	Glyceraldehyde 3-Phosphate Dehydrogenase
GI	Gene Index
GLUT	Glucose Transporter
GLP-1	Glucagon Like Peptide-1
GS	Goat Serum
GSIS	Glucose Stimulated Insulin Secretion
H	Hour
HbA1c	Glycosylated Hemoglobin
HBSS	Hanks' Balanced Salt Solution
HNF6	Hepatocyte Nuclear Factor- 6
HRP	Horseradish Peroxidase
IA-2	Insulinoma Associated Protein 2

IBMIR	Instant Blood-Mediated Inflammatory Reaction
IBMX	3-Isobutyl-1-Methyl Xanthin
ICC	Islet-Like Cell Cluster
IDDM	Insulin-Dependent Diabetes Mellitus
IDF	International Diabetes Federation
IEQ	Islet Equivalent
Ig	Immunoglobulin
IgG	Immunoglobulin G
IκB	Inhibitor of Kappa B Kinase
Ins	Insulin
IP	Intraperitoneal
IPF-1	Insulin Promoter Factor 1
IPGTT	Intraperitoneal Glucose Tolerance Test
IU	International Unit
kDa	Kilo Dalton
kg	Kilogram
L	Liter
LSD	Least Square Difference
LSM	Laser Scanning Microscopy
M	Molar
M	Marker
MEM	Minimum Essential Medium
mg	Miligram
MHC	Major Histocompatibility Complex
min	Minute
ml	Mililiter
mM	Milimolar
mm	Milimeter
MODY1	Maturity Onset Diabetes of the Young 1
MODY2	Maturity Onset Diabetes of the Young 2
MODY3	Maturity Onset Diabetes of the Young 3

MODY4	Maturity Onset Diabetes of the Young 4
mRNA	Messenger Ribonucleic Acid
N	Number
NAC	N-Acetyl-L-Cysteine
NAD	Nicotinamide Adenine Dinucleotide
ng	Nanogram
NHP	Non-Human Primate
Nkx2.2	NK2 Homeobox 2
Nkx6.1	NK6 Homeobox 1
nm	Nanometer
NO	Nitric Oxide
NOD	Non Obese Diabetic
PAGE	Polyacrylamide Gel Electrophoresis
PARP	Poly (ADP-ribose) Polymerase
PBS	Phosphate Buffer Saline
PBST	Phosphate Buffer Saline Tween
PCR	Polymerase Chain Reaction
PDX1	Pancreatic and Duodenal Homeobox 1
PI	Propidium Iodide
Pou3f4	POU Domain, Class 3, Transcription Factor 4
PP	Pancreatic Polypeptide
Ptf1a	Pancreas Specific Transcription Factor, 1a
PYY	Peptide Tyrosine Tyrosine
qPCR	Quantitative Polymerase Chain Reaction
R^2	Coefficient of Determination
RA	Retinoic Acid
Rb/Mo	Rabbit/Mouse
RGB	Red Green Blue
RLT	RNeasy Lysis Buffer
RNA	Ribonucleic Acid
RNase	Ribonuclease

Rpbjl	Recombination Signal Binding Protein for Immunoglobulin Kappa J Region-Like
RPE	RNEasy Principle
rpm	Revolution Per Minute
RQ	Relative Quantification
RT	Reverse-Transcriptase
RT	Room Temperature
RT-PCR	Reverse-Transcriptase Polymerase Chain Reaction
RT-real time PCR	Reverse Transcriptase Real Time Polymerase Chain Reaction
S.D	Standard Deviation
SDS	Sodium Dodecyl Sulphate
SDS-PAGE	Sodium Dodecyl Sulphate Polyacrylamide Gel Electrophoresis
sec	Second
SEM	Standard Error of Mean
SPSS	Statistical Program for Social Science
STZ	Streptozotocin
TAE	Tris Acetate Ethylenediaminetetraacetic
TBS	Tris Buffered Saline
TCM 199	Tissue Culture Medium-199
TEMED	tetramethylethylenediamine
TNE	Tris sodium chloride ethylenediaminetetraacetic
TNF	Tumor Necrosis Factor
TNF-α	Tumor Necrosis Factor Alpha
Tris HCl	Tris Hydrochloride
U	Unit
USD	United States Dollar
V	Version
V	Voltage
VEGF	Vascular Endothelial Growth Factor
vs	Versus
w/v	Weight/Volume

WHO	World Health Organization
Y	Y axis
Z	Z axis
Zn	Zinc

CHAPTER 1

INTRODUCTION

1.1 Background

Diabetes mellitus is a fast growing metabolic disease in the world (Kaul, 2013; WHO, 2013). In 2011, the world health organization (WHO) estimated more than 366 million people live with diabetes worldwide (Guariguata, 2012; WHO, 2013). However, this population is dramatically increasing and predicted to be more than double or 4.4 % of the total population by 2030. Diabetes is a disease that appears as a costly crisis for both developed and under developing countries (Guariguata, 2012). Worldwide healthcare costs of diabetes totaled USD 465 billion in 2011, equivalent to 11% of total health expenditure. In the United States alone, it costs the economy more than USD 100 billion annually (Guariguata, 2012). Diabetes causes to suffer its victims, their kidneys, eyes, hearts, limbs, spontaneity, and ultimately their lives. Approximately, in Malaysia it is estimated that 8.3% of the population are living with diabetes, of which 36% of the diabetic population have been undiagnosed with the disease (Bakri, 2007; WHO, 2013). Diabetes is a syndrome of metabolism, in which the body does not produce or possibly use insulin due to failure of the pancreas or the pancreatic cells, the major late subsequences of chronic pancreatitis (Malka *et al*., 2000; Margener and Baillie, 1997). Insulin is the most popular peptide hormone that adjusts the glucose entrance into the cells and controls the carbohydrates and fat metabolism, particularly the glucose to glycogen conversion. In diabetic people, the pancreas insulin production becomes less or failed or the body is not efficiently capable to use the secreted insulin. Subsequently, the glucose level in blood increased and cannot enter the cells to cause their growth. When, it reaches a maximum level glucose runs off into the urine and leaves the body. Therefore, the main source of body energy is lost even though huge quantity of glucose is present in the blood. Although genetics and environmental factors such as obesity and exercise deprivation are known as key role players of diabetes affection, the main causes of diabetes still remain mysterious.

The main types of diabetes are: Type 1 or juvenile diabetes, which is known as an autoimmune disease, when the body does not produce insulin and insulin-secreting cells in the pancreas are destroyed by an immune system attack. Consequently, the pancreas is not capable to produce insulin or secretes a little. Therefore, type 1 diabetic patients require daily insulin administration to live. These patients are not able to survive without insulin treatment. Majority type 1 diabetes patients are young, and the highest of onset is early at puberty. The first severe symptoms are polyuria, tiredness, thirst and weight-loss. Type 2 or adult–onset diabetes usually identified as a metabolic disorder that related to obesity, characterized by high blood pressure, and lipid level. In type 2 diabetes, which usually followed after the insulin resistance condition in the body where the pancreatic β-cells are either producing inadequate insulin or the cells are not able to use it. Accordingly, insulin secretion is reduced, blood glucose level builds up and the body failed to use glucose competently as its vital source of energy. Lastly, there is another type of diabetes that occurs in pregnant women, which is termed gestational diabetes. It is like type 2 diabetes and develops usually in people with a family history of diabetes. However, after delivery, it may disappear but the mother stands at a high risk of type 2 diabetes.

Numerous studies carried out on diabetes complications and treatment. Main progresses include the development of external insulin pumps without insulin injection requirement and pancreas transplantation which needs strong immunosuppressive drugs to protect the transplanted organ from rejection. Therefore, islet transplantation is the most promising treatment of type 1 diabetes and does not need an invasive surgery.

The islet transplantation subject has been considered for years. Just 1-2% of the whole pancreas is the islets of Langerhans. They resemble ellipsoid clusters with different diameters ranging from 50-500 µm containing approximately 1600-3000 cells (Beger *et al*., 2009). Pancreatic islets contain five different types of cells where the insulin secreting cells are the majority population basis of the pancreatic islets and species. The four other types of cells in an islet are α-cells which secrete glucagon, which causes to raise blood glucose level; δ-cells which secrete somatostatin that prevents the release of several hormones; and pancreatic polypeptide (PP) cells and D1 cells, about which little information is available (Beger *et al*., 2009).

Several factors have limited the success of islet transplantation. These factors are the activity variation of collagenase, the toxic factors for β-cell, the lack of correlation amongst islets yield and enzyme activity, and the collagenase effect on the immunogenicity and chemotactic performance of the islets (London *et al*., 1998). However, due to the availability of characterized collagenase preparations as well as validation and optimization of the isolation protocols, islet cells have been successfully transplanted (Shapiro *et al*., 2001).

Pancreatic islet transplantation is more considered as an alternative cure for type 1 diabetic patients (Markmann *et al*., 2003; Shapiro *et al*., 2000), although, achievement of normoglycemia needs a huge number of islets harvested from more than one pancreas donors. If one donor islet transplantation becomes evenly effective, it can resolve problem of a small population (0.5%) of type 1 diabetics, because human tissues for transplantation are not sufficient (Lakey *et al*., 2003). Thus, consideration of alternative islets sources appears to be critical. Pigs were considered as islet sources because at their rapid breeding, and good records of porcine insulin usage in humans and genetic engineering potential. However, immunologic complications should be considered and defeated, especially porcine islet damage by immune system and contact to blood factors (Bennet *et al*., 2000; Dorling *et al*., 2002). In primates, earlier studies showed that xenogeneic islets of Langerhans are at risk of instant damage after transplantation (Bennet *et al*., 2000). Contact of porcine islets to human serum or blood, even *in vivo* or *in vitro*, caused serious islet destruction principally arbitrated by complement (Bennet *et al*., 2000; Contreras *et al*., 2001; Contreras *et al*., 2004). Triggering of complement after xenoreactive antibodies which attached to the cell surface antigens on the graft is responsible for hyperacute graft rejection (Miyatake *et al*., 1998). Additionally, studies show that islets express a tissue factor, the main coagulation initiator *in vivo*. In islet transplantation, when islets directly interact with blood, the tissue factor of islet activates a damaging clotting reaction, related to ‘‘instant blood-mediated inflammatory reaction,’’ which is determined by initiation of the coagulation and complement systems, quick binding and platelet activation, and leukocyte

infiltration to the islets. These phenomena stimulate islet morphology destructions, islet dysfunction and death (Bennet *et al*., 2000).

1.2 Problem Statement

Diabetes is a silent and painful killer. Annually, diabetes reports show 4.6 million deaths worldwide and in some countries, shortage of insulin kills children and young people before being diagnosed. Diabetes is classified as the top ten causes of disability worldwide and undermines productivity and human development (WHO, 2013). Dead islets replaced with viable islets is a promising offer to restore normal insulin production to a person with diabetes. The main reason for establishing a new islet source for transplantation is the insufficiency of human donor pancreas whilst using xenogeneic islets perhaps assist this problem. Researchers have developed the porcine islet preparation protocols (Marigliano *et al*., 2011). However, several difficulties such as sensitivity and fragility of porcine islets are of concern as they can be easily be broken up, especially during the isolation and purification procedures, a less noticeable occurrence reported in islets from other species (Kim *et al*., 2007). The fragility of islets can lead to islet loss during culture, immunoalteration procedures, cryopreservation and banking (Swanson *et al*., 2001). Isolation and purification of caprine pancreatic islets were optimized in our laboratory (Hani *et al*., 2010; Vakhshiteh *et al*., 2013). Caprine pancreatic islet topography, culture and *in vivo* viability were evaluated as a new islet source consideration in diabetes research.

1.3 Objectives:

General:

To establish effective caprine pancreatic islets for xenotransplantation.

Specific:

1- To identify caprine pancreatic islets topography: the arrangement and distribution of α-, β- and δ-cells in caprine pancreatic islets,
2- To inspect the impact of antioxidant and secretagogues factors *in vitro* maintenance of caprine islets and their viability in culture media,
3- To scrutinize the *in vitro* enhancing factors for insulin promoter gene and protein expression in caprine islets,
4- To assess the functionality of grafted caprine islets in immunized diabetic murine models.

1.4 Null Hypothesis:

H0:

Caprine islets of Langerhans are not viable and do not have the insulin secretion ability throughout *in vitro* and *in vivo* trials

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