Review Article

Stem Cell Therapy to Restore Pancreatic Function in Dogs and Cats

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Abstract

Diabetes mellitus is a common disease in dogs and cats. It consists a group of metabolic diseases characterized by hyperglycemia resulting from defects in secretion and/or insulin activity. The islets of Langerhans from donor pancreas may be an alternative for the cure of diabetes, however, this approach is limited because the donation is scarce and complications occur due to the concurrent use of immunosuppressive drugs. For many decades researchers have sought ways to replace pancreatic islets in diabetic individuals. Current studies in progress with stem cell culture for production of pancreatic islet cells are promising, despite the difficulties in their production. This review reports several aspects concerning the use of stem cells in diabetes cell therapy. Recent studies in mice have shown that embryonic stem cells can be induced to differentiate into insulin-producing β-cells. In parallel with this study, a new class of stem cells has emerged, i.e. induced pluripotent stem cells (iPSCs) aimed at clinical and therapeutic use. Adult stem cells may circumvent the ethical issues surrounding embryonic stem cells and allow auto-transplantation.

Key words: Stem cell, diabetes, β-cell, pancreatic islets.

Introduction

Diabetes mellitus (DM) is an ubiquitous endocrine disease associated with absolute or relative insulin deficiency. This disease can affect dogs and cats and if it is not diagnosed and properly treated can be fatal (21, 26, 40). In dogs, DM can be classified into three types according to the secretory capacity of pancreatic β-cells. Group I or insulin dependent, caused by the destruction of β-cells with progressive cellular loss up to complete failure of insulin secretion. Group II or non-insulin dependent, is characterized by insulin resistance and/or dysfunctional β-cells. Insulin secretion can be high or just below normal, but is insufficient to overcome the insulin resistance in tissues. And Group III has been characterized in dogs which have a slightly elevated blood glucose concentration and some glucose intolerance but a basal concentration of insulin (27, 28). Diabetic animals can be considered as a model of human diabetes although animal diabetes has a wide variety of pathophysiological factors and occasionally does not correspond to human diabetes. Nonetheless, these animal models are useful for the study of acute and chronic complications of the disease (25).

In 1869 it was demonstrated the presence of Langerhans islets supplied by blood vessels, independent of the exocrine pancreas (20, 37). Lagues (1893) reported that two different groups of cells were responsible for the internal secretion of the endocrine pancreas, within the islets of Langerhans (41, 50). Diabetes in dogs was first described in 1889, when Merin and Minkowski reported this disease in pancreatectomized dogs (5, 40). According

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to Nogueira (28) the insulin secreted by β-cells is the main hormone involved in the syndrome of DM.

Several factors predispose to the development of DM: among the most important are the immune-mediated insulitis, pancreatitis, obesity, hormonal antagonism, use of certain drugs such as glucocorticoids, oral contraceptives, phenytoin, and injections of progesterone; genetic predisposition, infections, intercurrent illnesses (renal failure, heart disease) chronic relapsing pancreatitis, pancreatic trauma, idiopathic reduction of the number of functional β-cells, Cushing's syndrome, hyperreactive anterior pituitary or adrenal cortex, or some other factor that causes degeneration of the islets of Langerhans (10, 16, 28).

The human body is dependent on progenitor stem cells which frequently repair the specific cellular populations of the organs. Recently, several studies have shown the ability of endogenous stem cells from ischemic tissues to perform restorative functions that are decreased in patients with diabetes, and also in cardiovascular diseases in patients with DM type I, since β-cell destruction leads to dependence on insulin for glucose homeostasis (9, 11).

It is the aim of this paper to review the state of the art on diabetes and diabetes cell therapy.

Stem cells and diabetes

The pancreas, an organ composed of many cell types, consists of exocrine tissue containing cells which secrete enzymes into the intestine to assist in the digestion of food. Distributed throughout this exocrine tissue are thousands of islets of Langerhans, clusters of endocrine cells that produce and secrete hormones into the blood to maintain homeostasis. The insulin-producing β-cell is one type of endocrine cell in the islet; other types include alpha cells (α-cells), which produce glucagon, PP cells (γ-cells), which produce pancreatic polypeptide, and delta cells (δ-cells), which produce somatostatin (12). The embryonic origin of these pancreatic cells is controversial. According to Dor and Melton (7) during the embryonic development of the pancreas, β cells are generated from a transient population of endocrine progenitor cells expressing the transcription factor neurogenin3 (ngn3). Nevertheless, during the postnatal life, ngn3-positive progenitor cells disappear, and β cell expansion and maintenance relies on the proliferation of terminally differentiated β cells. Studies have indicated that human and rodent pancreatic duct cells, islet derived cells, and exocrine tissue have precursor cells that can differentiate toward β-cells (8).

DM is a group of metabolic diseases characterized by hyperglycemia resulting from defects in secretion and/or insulin action. According to the physiological and pathological changes that affect β-cells it receives a specific classification, but in some rare cases, β-cells are normal and diabetes results from suppression of insulin activity. When the pancreas is involved and has been primarily affected β-cells lose their ability to produce insulin; when the pancreas is secondarily affected by high blood glucose levels, histologically β-cells are hyperplastic (10, 28).

DM is classified into three types: type 1, type 2 and gestational diabetes in dogs and mice. Type 1 diabetes is an autoimmune syndrome, it is the most common in dogs and it is characterized by selective destruction of insulin-producing β-cells in pancreatic islets, mediated by T lymphocytes and macrophages, with progressive and eventually complete loss of insulin secretion. Therefore, patients that have this diabetes type are insulin-dependent. The etiologic and pathogenic bases of type 1 DM are dependent on genetic and environmental factors with more than twenty genes involved in the development of the disease (33).

DM type 2 is characterized by resistance to insulin action and is considered non-insulin dependent or from dysfunctional β-cells. Insulin secretion may be high, low or normal, but it is insufficient to overcome insulin resistance in tissues. This form of diabetes is difficult to identify clinically in dogs (4) and frequently is observed in middle-aged and older dogs (23). The strong polygenic inheritance associated with environmental factors such as obesity and physical inactivity are determinants in the genesis of type 2 DM (45).

Some of the most common complications associated with diabetes in dogs are neuropathies, glomerulopathy, retinopathy, and gastrointestinal disorders. Skin lesions associated with diabetes in dogs are uncommon but, when present, are associated with pyoderma, seborrheic syndrome, thinning and hyperpigmentation of the skin, and alopecia in several degrees (2).

The development of cataract is a common secondary complication of diabetes in dogs and is directly related to the severity and time of duration of hyperglycemia. During persistent hyperglycemia, glucose does not require insulin to enter the cells of the lens, overcoming the usual facilitated transport (34, 52). Other changes as oxidative stress within the organ are also involved (30). Cystitis is also a common complication in diabetes, for glucose in urine promotes growth of bacteria such as *Escherichia coli* and *Proteus* sp (34).

In cats, diabetes is also a relatively common endocrine disease. The incidence of type 2 DM may be as high as 50-70% in this species. Several risk factors were identified, like obesity, age, and sex and neutering process (31, 38). More than 50% of diabetic cats are older than 10 years; therefore, the age was identified as the most important risk factor. Obesity increases the risk of developing diabetes in 3 to 5 times. Neutered cats have almost as twice the risk, and males 1.5 times the risk of developing diabetes. Diabetes in young cats is extremely rare (35, 55).

Cats develop type 2 DM with the majority of diabetic cats developing islet amyloid (34), which is characteristic of this kind of diabetes in humans (48, 54). Two other findings support the notion that type 1 diabetes is probably rare in cats: antibodies against β-cells have not yet been documented (15) and lymphocytic infiltration of β-cell areas are extremely rare in diabetic cats (13).

Among treatment strategies for diabetes, we can mention pancreas transplantation, islet transplantation and, more recently, cell therapy using stem cells, to achieve insulin-independence for diabetes type 1 (8).

The transplantation of cells, tissues, and organs to restore physiological responses and/or anatomical structures has been the aim of medical studies. Thus, diabetes, in which the damage is localized to a specific cell type, seems to be a good candidate for cell therapy (4). In this way, studies with stem cells have increased in the last years in order to obtain different sources of cells which can be used for cell therapy. By definition stem cells are self-renewable progenitor cells that can be differentiated into one or more cell types. In addition, stem cells have an unlimited capacity of replication and, if properly treated, can differentiate in all cell types of the adult body. Basically, two types of stem cells are being investigated: embryonic (embryonic stem cells) and adult stem cells (22).

Parallel to the study of embryonic stem cells which show several ethical and religious problems in relation to their laboratory use, new classes of stem cells have emerged, aiming a clinical and therapeutic use: adult stem cells and induced pluripotent stem cells (iPSC). Adult stem cell is an indigenous undifferentiated cell found in specialized tissues in which it can replace damaged cells as required and ensure organ maintenance. In addition, in 2006 other type of pluripotent stem cells, i.e. the iPSC have been generated from mouse and human somatic cells by introducing Oct ¾ and Sox 2 with either Klf4 and cMyc or Nanog and Lin28 using retroviruses or lentiviruses (42). So, iPSCs are an artificial type of stem cells derived from a non-pluripotent cell, typically an adult somatic cell by inducing the expression of specific genes (30). These researches result from the constant search for new sources more efficient and acceptable for therapeutic use in regenerative medicine.

Adult stem cells are typically committed to the mature cells of the tissue in which they are located. Under specific conditions they present certain *plasticity*, according to the signals from the extracellular environment, property known as *transdifferentiation*. Adult stem cells can be maintained in culture for long periods of time, although this capacity is more limited compared to that of embryonic stem cells (22).

Adult stem cells from the bone marrow were the most studied. Currently, they are used clinically to restore various components of the blood and immune system. There are two types of stem cells in the bone marrow: 1. the progenitor hematopoietic cells that form blood and immune system, and 2. stromal mesenchymal stem cells (MSCs), which form bone, cartilage and fat. Normally, these cells are recruited for tissue replacement after injury and / or angiogenesis of several organs because they are able to differentiate into several cell types, including cardiomyocytes, vascular endothelial cells, neurons, hepatocytes, epithelial cells, and adipocytes. This multipotent ability for differentiation coupled with their ability for both self-renewal and regulation of the immune response, place the MSCs as potentially new therapeutic agents for the treatment of DM complications (12, 44). Xie et al. (51) described a therapeutic effect of pluripotent stem cells in diabetic animals. These authors induced insulin release in response to glucose stimulation in vitro. In diabetic mice they retained regulated secretion of insulin and decrease blood glucose levels.

According to Voltarelli et al. (47), hematopoietic stem cells transplantation in DM-1 has a potential to prevent total destruction of insulin-producing pancreatic cells as well as to induce significant clinical responses. The study by Voltarelli et al. (46) reported that high-dose immunosuppression followed by autologous non-myeloablative hematopoietic stem cell transplantation has shown good results in patients with early onset of the disease, reducing mortality and allowing insulin independence in most patients. Haller et al. (14) reported that the liver shares endodermal embryonic origin with the pancreas. It has been shown that hepatic oval cells can be manipulated to induce co-expression of insulin and glucagon and are considered liver stem cells due to the ability to differentiate into hepatocytes and bile duct cells. In the study by Yang et al. (52), with hepatic oval cells *in vitro* there were obtained clusters of cells similar to pancreatic islets cells that expressed various hormones of the endocrine pancreas, such as glucagon, pancreatic polypeptide and insulin. Preliminary results showed that the oval cell-derived islet cell-like clusters displayed the ability to reserve hyperglycemia in a diabetic NOD-scid mouse.

Some studies induced the generation of insulin-producing cells from embryonic stem cells, as well as showed that the introduction of the β-cell transcription factor Pax4 (3), improved the efficiency of generating insulin-producing cells. However, these cells produce low amounts of insulin, compared to β-cells. A number of researches in order to understand the development of stem cells culture to produce islets of Langerhans are promising and despite the difficulties of producing cells, previous results showed that future is not distant. Thus, it is possible to obtain viable cells to produce insulin to replace cells damaged by the immune system in type 1 diabetes. This cell therapy must be associated with blocking of the host immune reaction which may harm new and native cells (6, 8).

The main difficulty in these procedures is to induce differentiation of stem cells giving rise to cells able to produce insulin in amounts equivalent to those

produced by β-cells, and also differentiated stem cells that respond to the stimulation of glucose by releasing insulin (8). According to Goldthwaite (12) there is a possibility that β-cells can be regenerated by differentiation of endogenous stem cells, from the proliferation of available β-cells, or a combination of both mechanisms. β-cells appear during embryonic development, suggesting that their presence involves the temporal control of a number of genes.

Thomson et al. (43) described a method to isolate and cultivate human embryonic stem cells to treat type 1 diabetes. In theory, embryonic stem cells are able to differentiate into pancreatic β-cells to produce insulin. According to these authors, after being grown in vitro the necessary amount of insulin-producing cells for transplantation can be obtained. Thus cell therapy could be applied widely in diabetic patients. In addition, these cells can be manipulated to avoid immune rejection, even before transplantation: they could be packaged/wrapped in non-immunogenic and not rejected, freeing the patient from the devastating effects of immunosuppressive drugs. However there are some remaining problems concerning the use of embryonic cells, mainly related to ethical approaches and teratoma formation (32).

Most of the studies on regeneration analysis of β-cell have been conducted in animals. Rosenberg et al. (36) induced the regeneration of pancreatic islets, including β-cells by cellophane wrapping of the pancreas in hamsters. In this autoradiographic study, the regenerated β-cells showed insulin secretory capacity in vitro. According to Andersson et al. (1) when β-cells of pancreatic islets were cultured in a specific medium with high glucose concentration they showed extensive degranulation and increased amounts of rough-surfaced endoplasmic reticulum. The immunoreactive insulin content of islets cultured at 28 mM glucose was markedly decreased and the insulin secretion during culturing was much higher than with the islets cultured at 3.3 mM of glucose. These results showed that the biosynthesis of insulin proceeded at a high rate and remained regulated by glucose. Mesenchymal stem cells (MSC), also known as multipotent mesenchymal stromal cells, have been reported to secrete several cytokines, such as basic fibroblast growth factor (bFGF), vascular endothelial growth factor (VEGF), monocyte chemoattractant protein-1, and stem cell-derived factor-1, that exhibit angiogenic and neurosupportive effects (17, 18, 19). Since diabetic polyneuropathy is the most common complication of diabetes (49), Shibata et al. (39) demonstrated that transplantation of MSC in rats improved the hypoalgesia, delayed motor nerve conduction velocity, reduced sciatic nerve blood flow, and decreased axonal caliber in diabetic nerves of the treated limbs. The immunohistological study revealed that the capillary number–to–muscle fiber ratio was increased when MSCs were injected in the hind limbs and skeletal muscles. Four weeks after the transplantation,

MSC maintained their viability and secreted angiogenic factors such as VEGF and bFGF at the injected sites.

Mature β-cell itself has been studied as having an important role in proliferation. It is known that cyclin D2 is a protein involved in the process of replicating mature β-cells in the postnatal period (24). To test the role of this protein, Georgia and Bhushan (11) created genetically modified rats that did not express the gene for cyclin D2. Rats which do not express this gene, showed a lower rate of β-cell replication when compared to lower controls and all became glucose intolerant.

Regardless the severity of coronary artery disease, diabetic patients are at increased risk of developing heart failure. Diabetic cardiomyopathy (DCM) is characterized by microvascular pathology and interstitial fibrosis. Results showed that transplantation of mesenchymal stem cells improved cardiac function in a rat model for DCM, possibly through angiogenesis and attenuation of cardiac remodeling. Since MSCs are able to differentiate into cardiomyocytes and vascular endothelial cells, studies have shown that the transplantation of MSCs may inhibit apoptosis in the ischemic heart through upregulation of Akt (Protein Kinase B) and eNOS (Endothelial Nitric Oxide Synthase) and inhibit myocardial fibrosis in dilated cardiomyopathy, decreasing the expression of matrix metalloproteinases (MMP) in rat models (55).

Diabetic retinopathy is another common complication of diabetes, which is caused by injury to retinal microvasculature and neurons. The results of a current study suggested that adult MSCs may improve the integrity of the blood-retinal barrier in diabetic rats by differentiating into photoreceptor and glial-like cells in the retina and by reducing blood glucose levels (53).

Concluding remarks

Currently ongoing studies on stem cells for the production of pancreatic islet cells show promising results. It is still necessary to improve all protocols; there is strong evidence that stem cells are a potential source for tissue regenerative therapy. The differentiation of β-cells from stem cells is driven by non-completely known determinants, which include cell-cell interactions, extracellular matrix signals and the presence of a combination of growth factors, hormones, cytokines and nutrients. The main challenge is to obtain a cellular product able to mimic as closely as possible the basic features of a pancreatic β-cell to obtain a final cell product capable of restoring the loss of function in the pancreas.

Acknowledgements

References

1. ANDERSSON A, WESTMAN J, HELLERSTROM C. Effects of glucose on the ultrastructure and insulin biosynthesis of isolated mouse pancreatic islets maintained in tissue culture. **Diabetologia**, 1974, 10, 743-753.
2. ARIAS MVB, JERICÓ MM. Diabetes mellitus associado à dermatopatia em cão: Relato de caso. **Ciência Rural**, 1997, 27, 675-679.
3. BLYSZCZUK P, CZYZ J, KANIA G, et al. Expression of Pax4 in embryonic stem cells promotes differentiation of nestin-positive progenitor and insulin-producing cells. **Proc. Natl. Acad. Sci**. USA, 2003,100,998-1003.
4. CUNINGHAM JG. **Tratado de fisiologia veterinária**. 3. ed. Rio de Janeiro: Guanabara Koogan, 1996, p. 360.
5. DITTRICH HM. History of the discovery of pancreatic diabetes by von Mering and Minkowski 1889. A historical overview on the occasion of the 100th anniversary. **Z. Gesamte Inn. Med.,** 1989, 44, 335-340.
6. DOR Y, BROWN J, MARTINEZ OI, MELTON DA. Adult pancreatic β cells are formed by self – duplication rather than stem-cell differentiation. **Nature**, 2004, 429, 41-46.
7. DOR Y, MELTON DA. Facultative Endocrine Progenitor Cells in the Adult Pancreas. **Cell**, 2008, 132, 183-184.
8. EFRAT S. Generation of insulin-producing cells from stem cells for cell replacement therapy of type 1 diabetes. **Israel Med. Ass. J.**, 2004, 6, 1-3.
9. FADINI GP, SARTORE S, ALBIERO M, BAESSO I, MURPHY E, MENEGOLO M, GREGO F, KREUTZENBERG SV, TIENGO A, AGOSTINI C, AVOGARO A. Number and function of endothelial progenitor cells as a marker of severity for diabetic vasculopathy. **Arterioscler. Thromb. Vasc. Biol.**, 2006, 26, 2140 –2146.
10. FELDMAN EC, NELSON RW. 2004. **Canine and Feline Endocrinology and Reproduction**. 3. ed. Missouri: Saunders, 2004, 1089p.
11. GEORGIA S, BHUSHAN A. Beta cell replication is the primary mechanism for maintaining postnatal beta cell mass. **J. Clin. Invest.**, 2004, 114, 963-968.
12. **GOLDTHWAITE CA.** Are stem cells the next frontier for diabetes treatment. Regenerative medicine, 7, 2008. In *Stem Cell Information* [World Wide Web site]. Bethesda, MD: National Institutes of Health, U.S. Department of Health and Human Services, 2010 [cited Tuesday, July 10, 2012] Available at **http://stemcells.nih.gov/info/regenerative _medicine/2006chapter7**

13. HALL DG, KELLEY LC, GRAY ML, GLAUS TM. Lymphocytic inflammation of pancreatic islets in a diabetic cat. **J. Vet. Diagn. Invest.**, 1997, 9, 98-/100.
14. HALLER MJ, VIENER HL, WASSERFALL C, BRUSKOT, ATKINSON MA, SCHATZ DA. Autologous umbilical cord blood infusion for type 1 diabetes. **Exp. Hematol.**, 2008, 36, 710-715.
15. HOENIG M, REUSCH C, PETERSON ME. Beta cell and insulin antibodies in treated and untreated diabetic cats. **Vet. Immunol. Immunopathol.**, 2000, 77, 93-102.
16. IANUS A, HOLZ GG, THEISE ND, HUSSAIN MA. *In vivo* derivation of glucose-competent pancreatic endocrine cells from bone marrow without evidence of cell fusion. **J. Clin.Invest.**, 2003, 111, 843-50.
17. KINNAIRD T, STABILE E, BURNETT MS, EPSTEIN SE: Bone-marrow-derived cells for enhancing collateral development: mechanisms, animal data, and initial clinical experiences. **Circ. Res.**, 2004a, 95, 354 –363.
18. KINNAIRD T, STABILE E, BURNETT MS, LEE CW, BARR S, FUCHS S, EPSTEIN SE: Marrow-derived stromal cells express genes encoding a broad spectrum of arteriogenic cytokines and promote in vitro and in vivo arteriogenesis through paracrine mechanisms. **Circ. Res.**, 2004b, 94, 678–685.
19. KINNAIRD T, STABILE E, BURNETT MS, SHOU M, LEE CW, BARR S, FUCHS S, EPSTEIN SE: Local delivery of marrow-derived stromal cells augments collateral perfusion through paracrine mechanisms. Circ. Res., 2004c, 109, 1543–1549.
20. LEESON TS, LESSON R. Close association of centroacinar/ductular and insular cells in the rat pancreas. **Histol. Histopathol.**, 1986, 1, 33-42.
21. LING GV, LOWENSTINE LJ, PULLEY LT, KANEKO JJ. Diabetes mellitus in dogs. A review of initial evaluation, immediate and long term management and outcome. **J. AM. Vet. Med. Assoc**, 1977, 170, 521-530.
22. LOJUDICE FH, SOGAYAR MC. Células-tronco no tratamento e cura do diabetes mellitus. **Ciência e Saúde Coletiva**, 2008, 13, 15-22.
23. MARMOR M, WILLEBERG P, GLICKMAN LT, PRIESTER WA, CYPESS RH, HURVITZ AI. Epizootiologic pattern of diabetes mellitus in dogs. **Am. J. Vet. Res.**, 1982, 43, 465-470.
24. MATSUSHIME H, QUELLE DE, SHURTLEFF SA, SHIBUYA M, SHERR CJ, KATO JY. D-type cyclin-dependent kinase activity in mammalian cells. **Mol. Cell Biol.**, 1994, 14,, 2066-2076.
25. MÉNDEZ JD, RAMOS HG. Animal models in diabetes research. **Arch. Med. Res.**, 1994, 25, 367-75.
26. NELSON WN. Diabetes Mellitus. *In:* Birchard, S. J.; Sherding; R. G. Ed **Saunders manual of small**

animal practice. 18. ed. Philadelphia: Saunders , p.249-256, 1994.

27. NICHOLS R. Recognizing and treating canine and feline diabetes mellitus. **Vet. Med.**, 1992, 87, 211-222.

28. NOGUEIRA RB. Terapêutica do diabetes. *In:* Andrade, S. F. Ed. **Manual de terapêutica veterinária**. São Paulo: Roca, 2. ed., p. 331-345, 2002.

29. OBROSOVA IG, CAO X, GREENE DA, STEVENS MJ. Diabetes-induced changes in lens antioxidant status, glucose utilization and energy metabolism: effect of DL-lipoic acid. **Diabetologia**, 1998, 41, 1442-1450.

30. OKITA K, NAKAGAWA M, HYENJONG H, ICHISAKA T, YAMANAKA S. Generation of Mouse Induced Pluripotent Stem Cells Without Viral Vectors. **Science**, 2008, 322, 949-953.

31. PANCIERA DL, THOMAS CB, EICKER SW, ATKINS CE. Epizootiologic patterns of diabetes mellitus in cats: 333 cases (1980_/1986). **J. Am. Vet. Med. Assoc.**, 1990, 197, 1504-1508.

32. PROKHOROVA TA, HARKNESS LM, FRANDSEN U, DITZEL N, SCHRØDER HD, BURNS JS, KASSEM M. Teratoma formation by human embryonic stem cells is site dependent and enhanced by the presence of Matrigel. **Stem Cells Developm.**, 2009, 18, 47-54.

33. REDONDO MJ, EISENBARTH GS. Genetic control of autoimmunity in type I diabetes and associated disorders. **Diabetologia**, 2002, 45, 605-22.

34. REPETTI CSF, BORLINA AA. Homeopatia como tratamento adjuvante na diabetes mellitus canino (revisão de literatura). **Nosso Clínico**: São Paulo, 2003, 6 (33), 6-20.

35. ROOT MV, JONHSON KH, ALLEN WT, JOHNSTON SD. Diabetes mellitus associated with pancreatic endocrine insufficiency in a kitten. **J. Small Anim. Pract.**, 1995, 36, 416-420.

36. ROSENBERG L, DUGUID WP, VINIK AI. The effect of cellophane wrapping of the pancreas in the Syrian golden hamster: autoradiographic observations. **Pancreas**, 1989, 4, 31- 37.

37. SAKULA A. Paul Langerhans (1847-1888): a centenary tribute. **J. Royal Soc. Med.**, 1988, 81, 414 - 415.

38. SCARLETT JM, DONOGUE S. Associations between body condition and disease in cats. **J. Am. Vet. Med. Assoc.**, 1998, 212, 1725-1731.

39. SHIBATA T, NARUSE K, KAMIYA H, KOSAKE M, KONDA M, YASUDA Y, NAKAMURA N, OTA K, TOSAKI T, MATSUKI T, NATASHIMA E, HAMATA Y, OISO Y, NAKAMURA J. Transplantation of bone marrow-derived mesenchymal stem cells improves diabetic polyneuropathy in rats. **Diabetes** 2008; 57: 3099–3107.

40. SOSKIN S. The utilization of carbohydrate by totally depancreatized dogs receiving no insulin. **J. Nutr.**, 1930, 3, 34-40.

41. SPAGNOLI FM. From endoderm to pancreas: a multistep journey: Review. **Cell. Mol. Life Sci.**, 2007, 67, 2378-2390.

42. TAKAHASHI K, YAMANAKA S . Induction of Pluripotent Stem Cells from Mouse Embryonic and Adult Fibroblast Cultures by Defined Factors. **Cell**, 2006, 126, 663–676.

43. THOMSON JA, ITSKOVITZ-ELDOR J, SANDER SS, WAKNITZ MA, SWIERGIEL JJ, MARSHALL VS, JONES JM. Embryonic Stem Cell Lines Derived from Human Blastocysts. **Science**, 1998, 282, 1145-1147.

44. VOLAREVIC V, ARSENIJEVIS N, LUKIC ML, STOJKOVIC M. Concise Review: Mesenchymal stem cell treatment of the complications of diabetes mellitus. **Stem Cells**, 2011, 29, 5-10.

45. VOLTARELLI JC, COURI CEB, RODRIGUES MC, MORAES DA, STRACIERI ABPL, PIERONI F, NAVARRO G, MADEIRA MIS, SIMÕES BP. Terapia celular no diabetes mellitus. **Rev. Bras. Hematol. Hemoter.**, 2009, 31(1), 149-156.

46. VOLTARELLI JC, COURI CEB, STRACIERI ABPL, OLIVEIRA MC, MORAES DA, PIERONI F, COUTINHO M, MALMEGRIM KCR, FOSS-FREITAS MC, SIMÕES BP, FOSS MC, SQUIERS E, BURT RK. Autologous Nonmyeloablative Hematopoietic Stem Cell Transplantation in Newly Diagnosed Type 1 Diabetes Mellitus. **Jama**, 2007, 297 (14), 1568- 1576.

47. VOLTARELLI JC. Transplante de células tronco hematopoéticas no diabete melito tipo I. **Rev. Bras. Hematol. Hemoter.**, 2004, 26 (1), 43-45.

48. WESTERMARK P, WILANDER E. The influence of amyloid deposits on islet volume in maturity onset diabetes mellitus. **Diabetologia**, 1978, 15, 417-421.

49. WONG MC, CHUNG JW, WONG TK: Effects of treatments for symptoms of painful diabetic neuropathy: systematic review. *BMJ* 335:87, 2007 2. Vinik AI, Park TS, Stansberry KB, Pittenger GL: Diabetic neuropathies. **Diabetologia**, 2000, 43:957–973.

50. WOODS JP, PANCIERA DL, SNYDER PS, JACKSON MW., SMEDES SL. Diabetes mellitus in a kitten. **J. Am. Anim. Hosp. Assoc.**, 1994, 30, 177-180.

51. XIE QP, HUANG H, XU B, DONG X, GAO SL, ZHANG B, WU YL. Human bone marrow mesenchymal stem cells differentiate into insulin-producing cells upon microenvironmental manipulation in vitro. **Differentiation**, 77, 2009, 483-491.

52. YANG L, LI S, HATCH H, AHRENS K, CORNELIUS JG, PETERSEN BE, PECK AB. *In vitro* trans-differentiation of adult hepatic stem cells

into pancreatic endocrine hormone-producing cells. **Proc. Natl. Acad. Sci.**, 2002, 99, 8078-8083.

53. YANG Z, LI K, YAN X, DONG F, ZHAO C. Amelioration of diabetic retinopathy by engrafted human adipose-derived mesenchymal stem cells in streptozotocin diabetic rats. **Graefes Arch. Clin. Exp. Ophthalmol.,** 2010, 248, 1415–1422.

54. YANO BL, HAYDEN DW, JOHNSON KH. Feline insular amyloid: Association with diabetes mellitus. **Vet. Pathol.**, 1981, 18, 621-627.

55. ZHANG N, LI J, LUO R, JIANG J, WANG JA. Bone marrow mesenchymal stem cells induce angiogenesis and attenuate the remodeling of diabetic cardiomyopathy. **Exp Clinical Endocrinol. Diabetes,** 2008; 116: 104–111.