The Beneficial Impact of Stem Cloning on Diabetic Patients and How its Efficiency can be Improved

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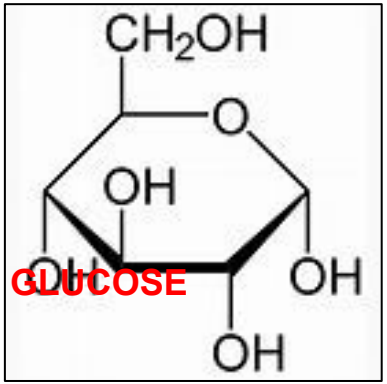
Overview of Diabetes -Background Research

- Diabetes refers to a group of diseases which impact our body's usage of **glucose** (blood sugar).
- Glucose is fundamental to human health because its a source of energy for our brain and the cells that compose our bodily tissues and muscles.
- The underlying cause of diabetes varies by type; however, ultimately, they all lead to an **excess amount of sugar in your bloodstream.** This can result in severe health complications.
- Type 1 and Type 2 diabetes are both **chronic** conditions, while prediabetes (blood sugar level is high, but not high enough to be classified as diabetes) is a **reversible** condition.
- Most often, prediabetes precedes diabetes, but progression can be prevented through appropriate measures.

Blood sugar testing provides very beneficial information for diabetic patients such as the effectiveness of certain medication.

The Critical Role of Insulin and Glucose in Our Body -Background Research

- **Glucose** is a simple sugar which acts as a energy source for our cells which compose bodily tissues and other organs. It is a fundamental component for cellular respiration: $C_6H_{12}O_6 + O2 \rightarrow$ energy + H20 + CO2.
- The two main sources of glucose in our body are the liver and the food we consume.
- The liver produces glucose at a stable rate through processes such as glycogenolysis and gluconeogenesis. However, the liver's glucose production is influenced by the amount of insulin available as well as other nutrients in the body.
- **Insulin** is a hormone secreted by the pancreas which releases it into our bloodstream. It circulates thus allowing glucose to enter our cells. It also regulates glucose levels in our bloodstream. However, if your blood sugar level decreases, so will the production of insulin in the pancreas.
- A lack of insulin triggers various forms of diabetes.

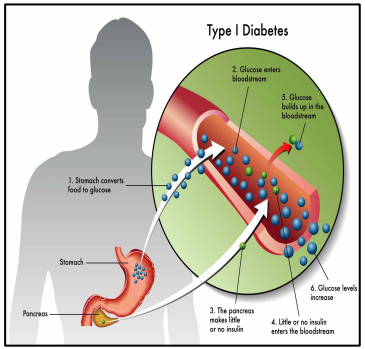


Type 1 Diabetes -Background Research

- **Type 1 diabetes** is a long-term condition in which the pancreas creates minimum amounts of insulin.
- There are various factors which contribute to type 1 diabetes, such as genetics or viruses. Despite the research dedicated to type 1 diabetes, this chronic condition has **no cure.** However, treatment aims to prevent further complications by managing glucose levels in the bloodstream via insulin.
- The primary cause of type 1 diabetes is undetermined, however, the most common occurrence leading to type 1 diabetes is when the body's immune system mistakenly eliminates insulin producing cells situated in the pancreas.
- Type 1 diabetes can develop at any age, but it is often developed during childhood/adolescence years.

Some symptoms of type 1 diabetes appear earlier on than others:

- An increase in thirst
- Continual urination
- Increased hunger
- Inexplicable mood changes
- Weight loss
- Fatigue
- Hazy vision



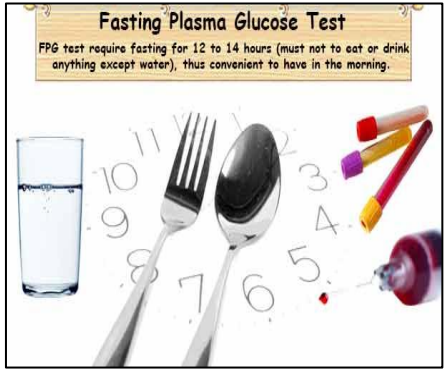
Prediabetes and Type 2 Diabetes -Background Research

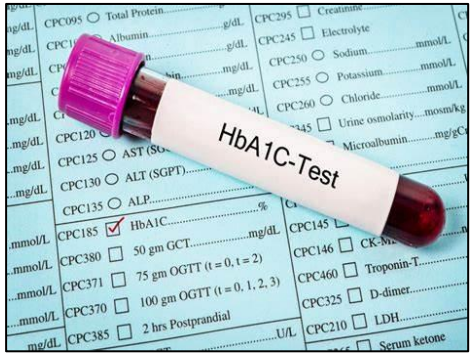
- **Prediabetes** refers to high concentration levels of glucose in the bloodstream. However, the blood sugar level isn't excessive enough to be identified as type 2 diabetes.
- If you are diagnosed with prediabetes, long-term complications of diabetes may already be affecting your body. However, progression from prediabetes to type 2 diabetes can be avoided.
- The primary cause of prediabetes is undetermined, however, genetics and being overweight are aspects which may contribute to the development of prediabetes.

- **Type 2 diabetes** results in excessive amounts of sugar in the bloodstream. This can lead to further complications of the circulatory and nervous systems.
- Potential problems leading to type 2 diabetes can be that the pancreas is not producing the required amount of insulin needed, or that the cells are not taking in as much sugar.
- This condition is **incurable**, although staying active, eating healthy, and other various methods can help you manage the disease.
- **Symptoms:** Increase in thirst, constant urination, increase in hunger, continual infections, numbness in certain parts of the body, darkened skin, etc.

Diabetes Diagnostic Methods -Background Research

- Most commonly health care professionals diagnose patients with diabetes using the (FPG) or the (A1C) test.
- **Fasting plasma glucose (FPG) test:** The fasting plasma glucose (FPG) test for short, measures the concentration levels of glucose in the bloodstream at a specific time. It is recommended to take the FPG test during the morning, after fasting for 8 hours. When fasting, it is advisable to not eat or drink anything other than water.
- **A1C test:** The A1C test is also known as the hemoglobin A1C test, measures the average amounts of glucose in your bloodstream over the course of 3 months. Eating and drinking before this test is not restricted. When using this test, doctors are required to confirm certain factors such as your age or whether or not you have anemia (a condition where you lack enough red blood cells). People diagnosed with anemia won't receive accurate results from this test.

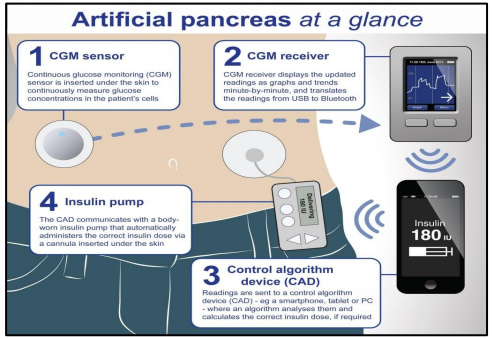




Current Treatment for Type 1 Diabetes -Background Research

- Although there is no determined cure for type 1 diabetes, there are a number of treatments that can potentially help you manage the disease. The general goal for treatment is to maintain glucose levels in your bloodstream.
- **Taking Insulin:** There are various types of insulins such as short-acting insulin, rapid-acting, and long-acting insulin etc. Insulin needs to be taken through injections or an insulin pump.
- **Artificial Pancreas:** Artificial pancreas also referred to as closed-loop insulin delivery is an implanted device connected to a glucose monitor. The device monitors the level of glucose continuously and provides the body with the needed amounts of insulin when it is required.

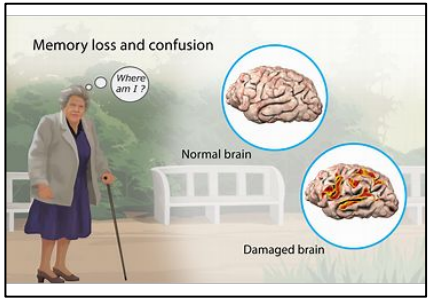
Insulin Injections



Artificial Pancreas

Long-term Health Complications and Risk Factors - Background Research

- The risk factors for diabetes vary with the type. However, common risk factors include family history (parent/sibling carrier), being exposed to viruses, presence of autoantibodies, weight for Type 2 diabetes (increased number of fatty tissues will make cells more resistant to insulin), inactivity, high blood pressure, age, etc.
- Long-term health complications develop slowly but have the potential to be disabling or even life-threatening. Common complications include cardiovascular problems such as coronary artery disease, stroke, or atherosclerosis, nerve damage (an excessive amount of sugar can lead to the destruction of capillary walls that nourish nerves) that if left untreated, can result in loss of sensation, kidney failure/irreversible kidney disease (requires dialysis or a kidney transplant), damage to retina blood vessels (can lead to blindness), hearing impairment, depression, and Alzheimer's disease.

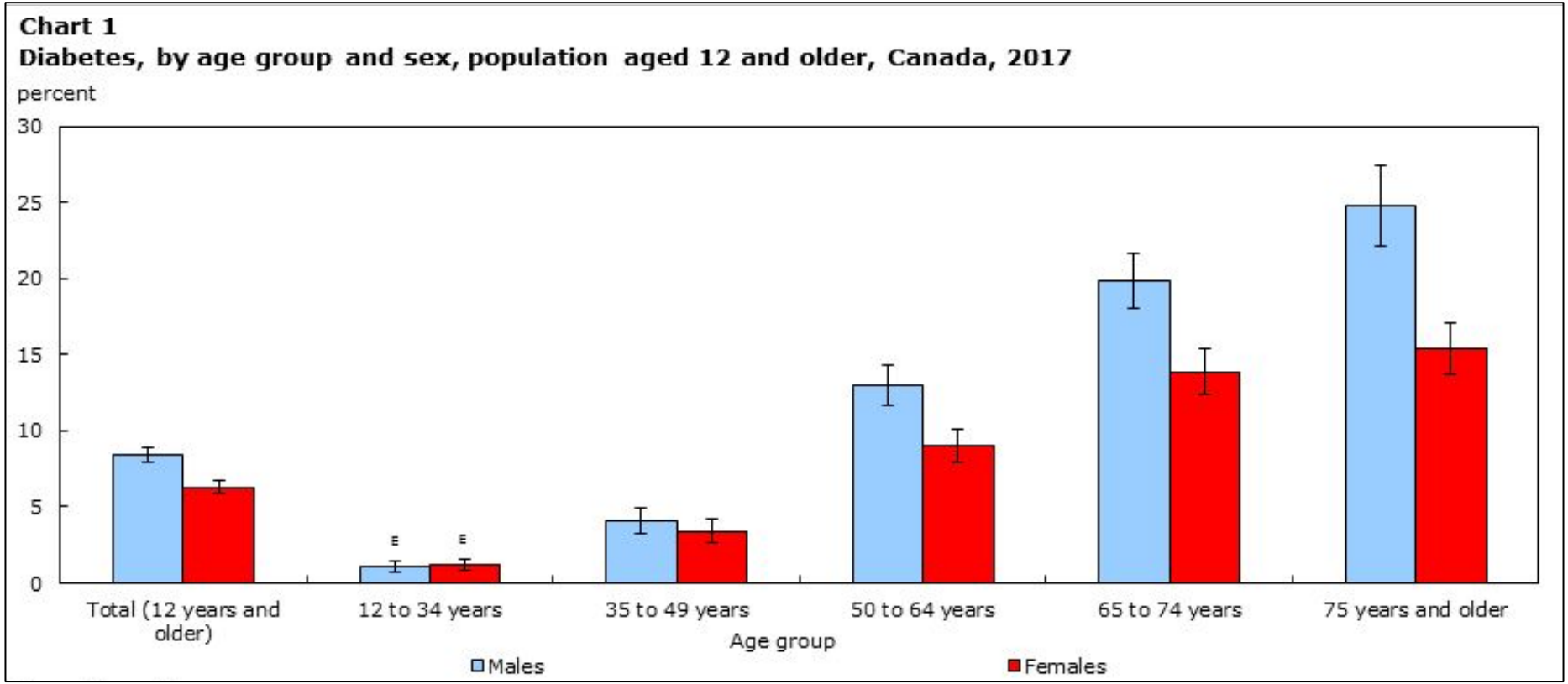


A Statistical Analysis - Background Research

According to Statistics Canada 2018 (Health Fact Sheets):

- "In 2017, 7.3% of Canadians aged 12 and older (roughly 2.3 million people) reported being diagnosed with diabetes" (Statistics Canada).
- "Canadians with type 1 diabetes have been living with their diagnosis for an average of 20.2 years, compared to 12.2 years for type 2 diabetes" (Statistics Canada).
- Overall, males were more likely than females to report a diabetes diagnosis (Statistics Canada).
- Diabetes increased with age for males, with the highest prevalence among those 75 years and older. For females, the percentage of those who reported a diagnosis of diabetes increased with age up to the age of 64. Prevalence among those 75 years and older did not increase (Statistics Canada).

Source: *Canadian Community Health Survey, 2017*



Source: *Canadian Community Health Survey, 2017*

Problem/Testable Question Investigated

Problem/Testable Question: *"Can stem cloning offer a potential cure for diabetic patients? If so, how could cloning efficiency be increased to support a higher success rate?"*

- As evidenced by the statistics in the previous slide, thousands of people are detrimentally affected by diabetes each year. Additionally, many people develop long-term health complications that can be disabling or even life-threatening.
- According to many studies that were conducted in 2016 and stem cell research, cloned human stem cells could provide a cure for those with Type 1 diabetes. Although small clinical trials are currently being carried out using stem cloning, the efficacy of the treatment on a large number of people is unknown. Some significant factors that prevent stem cloning to be used more practically is efficiency rate, effectiveness, and ethical concerns.
- We will briefly be exploring ethical concerns and discussing how stem cloning efficiency can be improved to benefit Type 1 diabetic patients. Stem cell research however, can be used for many more practical purposes other than diabetes treatment.

Formulating a Hypothesis

Problem/Testable Question: *"Can stem cloning offer a potential cure for diabetic patients? If so, could cloning efficiency be increased to support a higher success rate?"*

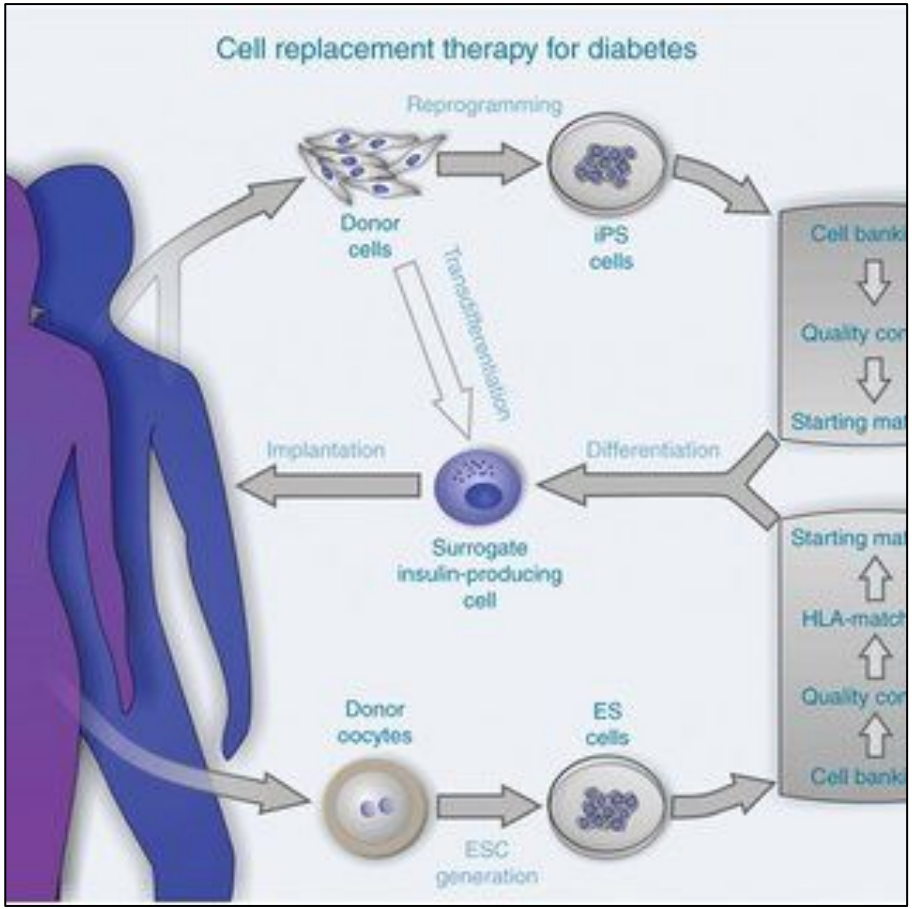
- Cloning is a special technology that ultimately, enables scientists to produce exact genetic copies of living organisms. Cells, specific genes, as well as entire animals have the ability to be cloned.
- Cloning is present naturally as well. For example, bacteria and other single-celled organisms are able to make exact copies of themselves every time they reproduce. Identical twins are a humane example of natural cloning. Twins (fertilized egg is split into two halves) share almost exactly the same genes.
- Hypothesis: If stem cloning efficiency is increased, then stem cloning could provide a potential cure for diabetic patients, because stem cells can be grown in labs and modified to become islet cells (groups of endocrine cells scattered throughout a tissue in the pancreas responsible for secreting insulin and glucagon) that can produce insulin (these cells can be transplanted into someone with Type 1 diabetes).

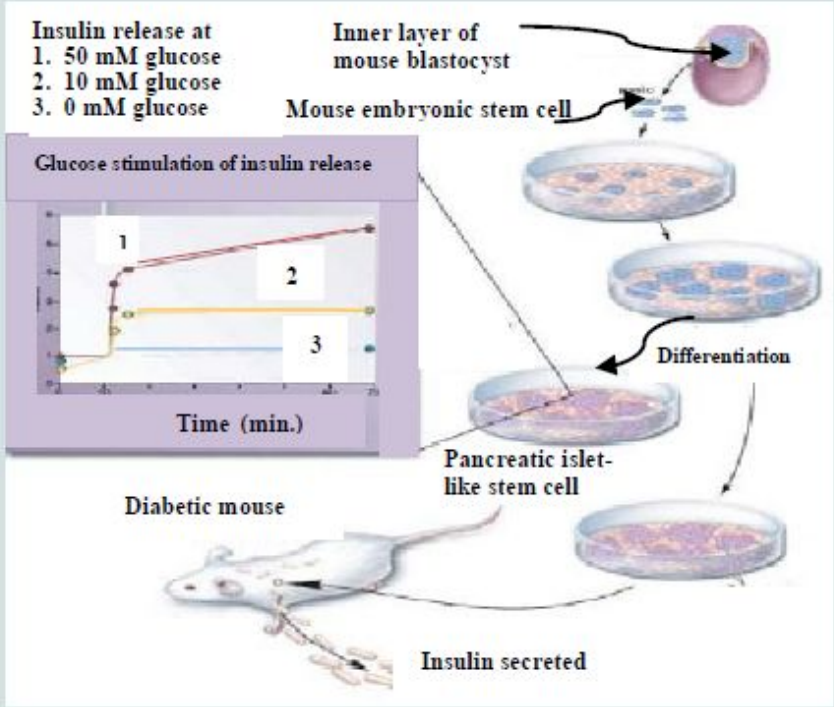
General Stem Cell Research

- **Stem Cells** are the cells from which specialized cells are produced. Stem cells often divide to form more cells, identified as “daughter cells.” These daughter cells now become specialized cells, for varied functions such as blood cells, nerve cells, muscle cells, etc. Stem cells are the only cells in the body capable of generating new specialized cells.
- **Where do stem cells originate from?** Over the years, researchers have determined several sources from which stem cells originate from.
- **Embryonic stem cells:** Embryonic stem cells develop from embryos (“unborn offspring in the process of development”) usually embryos that are 3-5 days old. Embryonic stem cells are very versatile and have the potential to divide and become various types of cells in the body. Since embryonic stem cells are pluripotent, they can be utilized to regenerate damaged tissue or organs in the body.
- **Adult stem cells:** The ability to regenerate and become into various types of cells in the body is more limited in adult stem cells than embryonic stem cells. These stem cells are situated in adult tissues such as bone marrow. Researchers presumed that adult stem cells situated in the bone marrow could become blood cells only, however, suggestive evidence says otherwise. Scientists have now succeeded in altering adult stem cells to have properties similar to embryonic stem cells by modifying their genes.

Curing Type 1 Diabetes Via Stem Cloning

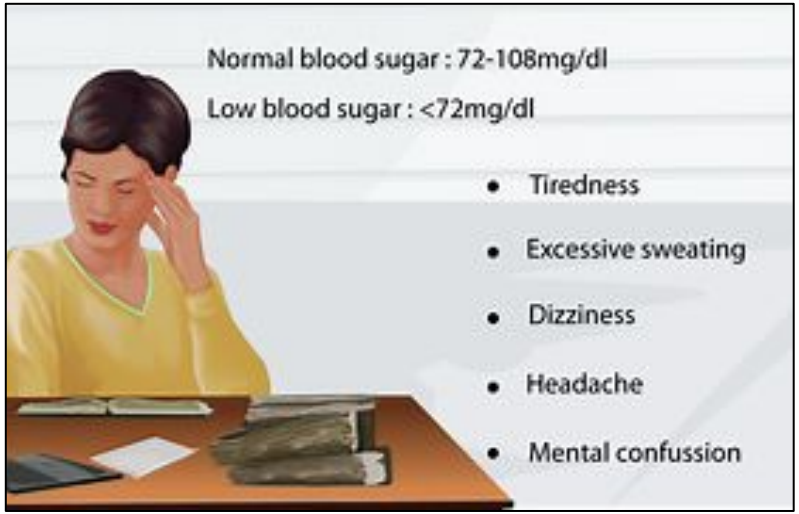
- Type 1 diabetes occurs in an individual when their immune system attacks the beta cells in the islets of the pancreas. These specific islets are responsible for producing insulin, and they are known as islet cells. Many researchers believe that these damaged islet cells can be replaced with healthy cells. However, this requires a method to be developed in which the individual's immune system doesn't attack the new cells. Essentially, this is the basic cure treatment for Type 1 diabetes. This is where stem cloning can be applied.
- Stem cells have not yet developed into the mature cells that compose our tissues and organs such as the cells in the heart, bones, brain, etc. In fact, they only form the cluster of cells in the body that have the ability to turn into one or another of these specialized cells. However, to do this, they must receive special instructions. An example of this is that a stem cell could become a new blood cell with proper biological instructions as well as in the ideal conditions.
- To produce cell therapies such as VX-880, scientists grow stem cells in labs. Then, they give proper biological instruction to become islet cells, which have the ability to produce insulin. These cells are then transplanted into someone who has been diagnosed with T1D.

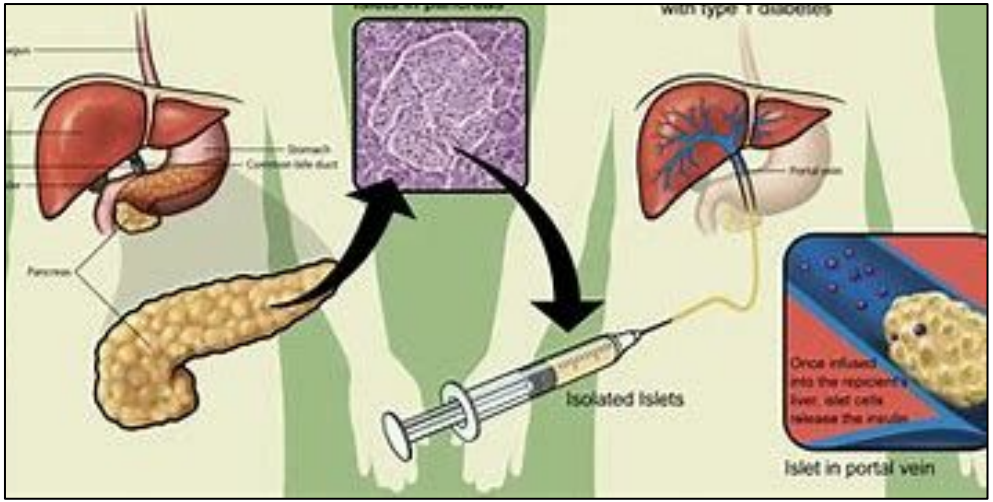

Cell replacement therapy for diabetes
Reprogramming
Donor cells
iPS cells
Transdifferentiation
Cell bank
Quality con
Starting mat
Implantation
Differentiation
Surrogate insulin-producing cell
Starting mat
HLA-match
Quality con
Cell bank
Donor oocytes
ES cells
ESC generation


Insulin release at
1. 50 mM glucose
2. 10 mM glucose
3. 0 mM glucose
Inner layer of mouse blastocyst
Mouse embryonic stem cell
Glucose stimulation of insulin release
1
2
3
Time (min.)
Differentiation
Pancreatic islet-like stem cell
Diabetic mouse
Insulin secreted

Curing Type 1 Diabetes Via Stem Cloning (Cont.)

- This treatment can ultimately restore an individual to their initial healthy state.
- Since 2016, scientists have known that islet transplantations can effectively cure diabetes. However, insufficient donor material has been a continuous issue.
- VX-880 Therapy: This was the first stem-cell-derived therapy that was found successful in clinical trials and could be offered as a treatment to T1D patients that are diagnosed with severe hypoglycemia (blood sugar levels drop below 4 mmol/L).
- The VX-880 therapy requires a person to also receive immunosuppressive therapy. This is because the stem cells are not derived from the same individual. The immunosuppressive therapy will basically "turn off" the individual's immune system so that it does not attack the new cells that were implanted. A similar procedure is undergone by individuals who get organ transplants.
- The first clinical trial/study that proved scientists' theory of islet transplantations being successful in treating diabetes was conducted in 2016.

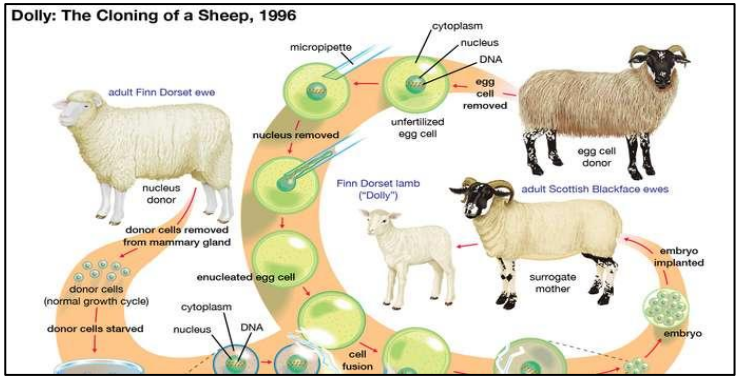

Normal blood sugar : 72-108mg/dl
Low blood sugar : <72mg/dl
Tiredness
Excessive sweating
Dizziness
Headache
Mental confussion

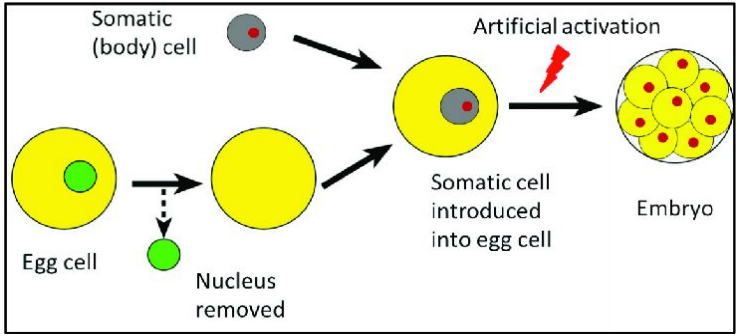

Isolated Islets
Islet in portal vein


Immunosuppressant
Drugs
© www.medindia.net

How Does SCNT Work?

- SCNT (Somatic Cell Nuclear Transfer) refers to the method in which a somatic cell's nucleus is moved to an enucleated egg (an egg without its nucleus). The somatic cell's nucleus is then modified into a zygote (fertilized egg) nucleus as it enters the cytoplasm of the enucleated egg. The egg then moves onto the blastocyst (embryo phase) stage. Once this occurs, embryonic stem cells can be formed from the blastocyst. Embryonic stem cells of monkeys and humans have been created through the process of SCNT. The basis of SCNT is utilizing an egg cell's cellular environment to transfer the cell nucleus into an embryonic state. This allows the animal undergoing the SCNT process to become genetically identical to the nucleus donor.
- Although SCNT has a lot of drawbacks including a low-efficiency rate, it proved its potential when the first mammal Dolly the sheep was cloned using SCNT.





General Cloning History Timeline

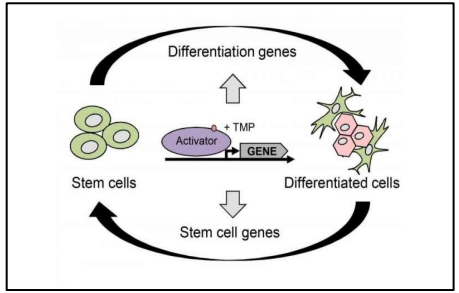
- **October 1990-** The National Institute of Health launched the Human Genome Project.
- **1993-** Calves were created by the transfer of nuclei of embryonic cells.
- **Later 1993-** Human embryos became newly cloned.
- **July 1995-** Megan and Morag (sheep) were cloned.
- **February 1997-** Dolly the Sheep was born.
- **July 1997-** The same scientists who made Dolly made Polly (Pall Dorset lamb).
- **August 1997-** President Clinton proposed legislation that would ban human cloning for five years.
- **January 1998-** Nineteen European nations banned human cloning as well.
- **January 1998-** The FDA officially announced its authority over human cloning.
- **Now-** What's next?

Ethical Concerns

- There are many ethical questions and concerns involved with stem cell research.
- Stem cell research has the potential to to treat people with many destructive diseases other than diabetes, such as Parkinson's, spinal cord injuries, and much more. Another example would be injuries associated with the nervous system. This is because stem cells can successfully maintain the function of damaged tissues and other cells. Nonetheless, there are many ethical questions associated with stem cell research. One of the major ethical concerns revolves around the methods which are used to obtain embryonic stem cells that in turn destroy the embryo.
- Some people believe that although an embryo is still under development, it is essentially a potential person. The argument there is that the embryo should be treated respectfully because it is technically still a human, even if it doesn't have characteristics yet. The counterargument is that an embryo is just an organic material similar to our body parts, until and unless it can survive on its own.

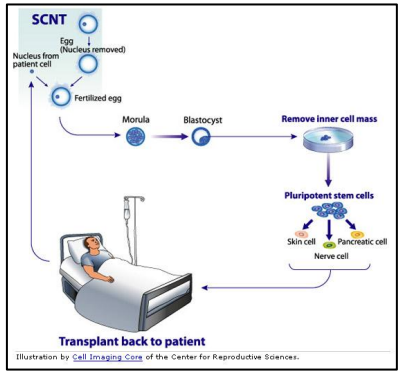
Low Efficiency Rate of Cloning

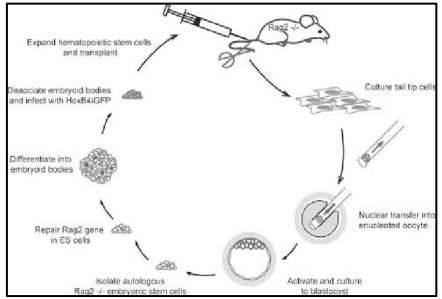
- SCNT is a technique with the potential to make many breakthroughs in science, however, the efficiency of this technique is very low. An example of this is Dolly the sheep. Although she was cloned using SCNT, it took the scientists 277 tries. Currently, the efficiency rate of SCNT in mice is 1 to 2 percent, 1 to 20 percent in cows, and even less in other species. This is because there are many barriers in SCNT preventing cloning to prove successful.
- **Gene Activation:** For an embryo to develop, genes need to be activated. Yi Zhang conducted an experiment and found out that in normal embryo development genes were successfully activated, whereas the genes in SCNT embryos failed to activate. In contrast to IVF (normal development), 222 regions in the cell undergoing the process of SCNT had genes that were inactive. Because of this, the chances of an embryo developing completely through SCNT are smaller, hence the low-efficiency rates.



How can the Efficiency Rate of Stem Cloning be Improved?

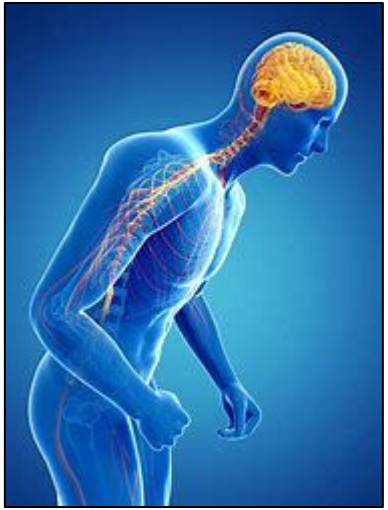
- Stem cloning efficiency rates are low due to certain barriers in the SCNT method preventing successful cloning. One of the factors contributing to the low-efficiency rate of SCNT is gene activation. Through an experiment conducted by Yi Zhang, he found that many embryonic genes in the donated nucleus were inactivated. After further research, he concluded that it was due to an epigenetic roadblock. This roadblock preventing the genes to activate was a protein that packaged the DNA inside the cell. After removing this, he was able to raise the SCNT efficiency from 1 to 2 percent, to 8 to 9 percent.
- Later, Zhang conducted the same experiment but this time with human SCNT. They decided to use the same method they did with the previous experiment, where they were able to improve SCNT efficiency by removing tags that kept genes in the donated nucleus inactivated. To do this, RNA was injected into the cell to form an enzyme capable of removing tags preventing the genes from activating. The result was similar to the previous experiment, and the SCNT embryo now had a 14 percent success rate of making it to the blastocyst stage.





Conclusion

- Our hypothesis was correct. Stem cloning can be used to potentially cure patients with TD1. More specifically, though, it is islet transplants that can provide a cure for Type 1 diabetes.
- However, there are only small clinical trials being carried out using this treatment. This is due to factors such as ethical concerns, low efficiency rate, and insufficient donor material.
- Although efficiency rates of stem cloning are low, conducted experiments show that by eliminating certain roadblocks that prevent SCNT from being successful, we can possibly increase these efficiency rates.
- Stem cloning is a vast area of research. In addition, it has the potential to treat many other degenerative diseases other than diabetes. For example, it could be used to potentially treat Parkinson's, nervous system injuries, spinal cord injuries, etc.
- If factors such as low efficiency rate are resolved completely, stem cloning could one day be used practically as a treatment for a larger population of society.



Bibliography- Research Sources

- Friedman, Author Janice. “Stem Cell Article.” *BIOLOGY JUNCTION*, 29 Apr. 2019, www.biologyjunction.com/stemcell_article.htm.
- *Transfer and Cloning of the Insulin Gene - Access Excellence*. accessexcellence.org/RC/VL/GG/transfer_and.html.
- “Type 1 Diabetes.” *Mayo Clinic*, Mayo Foundation for Medical Education and Research, 22 Aug. 2020, www.mayoclinic.org/diseases-conditions/type-1-diabetes/symptoms-causes/syc-20353011.
- “Diabetes.” *Mayo Clinic*, Mayo Foundation for Medical Education and Research, 30 Oct. 2020, www.mayoclinic.org/diseases-conditions/diabetes/symptoms-causes/syc-20371444.
- “Type 2 Diabetes.” *Mayo Clinic*, Mayo Foundation for Medical Education and Research, 20 Jan. 2021, www.mayoclinic.org/diseases-conditions/type-2-diabetes/symptoms-causes/syc-20351193.
- “Somatic Cell Nuclear Transfer.” *Encyclopædia Britannica*, Encyclopædia Britannica, Inc., www.britannica.com/science/somatic-cell-nuclear-transfer.
- “What Is Cloning, and What Does It Have to Do with Stem Cell Research?” *Eurostemcell*, 29 Mar. 2007, www.eurostemcell.org/what-cloning-and-what-does-it-have-do-stem-cell-research#:~:text=General%20Stem%20Cell%20Knowledge%20Cloning%2C%20or%20somatic%20cell,the%20nucleus%20of%20a%20cell%20from%20another%20adult.
- “Blastocyst.” *Encyclopædia Britannica*, Encyclopædia Britannica, Inc., www.britannica.com/science/blastocyst.
- “Clinical Trial Tests Stem Cell Therapy to Cure People with Type 1 Diabetes.” *DiaTribe*, 16 Mar. 2021, diatribe.org/clinical-trial-tests-stem-cell-therapy-cure-people-type-1-diabetes.

Bibliography- Research Sources (Cont.)

- Ulrich, Tom. “Improving Cloning Efficiency.” *Improving Cloning Efficiency | Harvard Medical School*, 6 Nov. 2014, hms.harvard.edu/news/improving-cloning-efficiency.
- Ulrich, Tom. “Another Hurdle Cleared.” *Another Hurdle Cleared | Harvard Medical School*, 6 Nov. 2015, hms.harvard.edu/news/another-hurdle-cleared.
- “Diabetes Tests & Diagnosis.” *National Institute of Diabetes and Digestive and Kidney Diseases*, U.S. Department of Health and Human Services, 1 Dec. 2016, www.niddk.nih.gov/health-information/diabetes/overview/tests-diagnosis#diagnosediabetes.
- “Frequently Asked Questions about Stem Cell Research.” *Mayo Clinic*, Mayo Foundation for Medical Education and Research, 8 June 2019, www.mayoclinic.org/tests-procedures/bone-marrow-transplant/in-depth/stem-cells/art-20048117.

Bibliography- Online Image Sources

https://www.researchgate.net/figure/Somatic-cell-nuclear-transfer-SCNT-SCNT-involves-the-removal-of-the-chromosomes_fig3_285619276

https://www.pinterest.ca/pin/761108405748752535/

http://cysticfibrosis.com/artificial-pancreas/

https://phys.org/news/2015-09-method-gene.html

233826a67be66a810b23a263230da62e_XL.jpg (900×878) (care4heart.com)

http://www.biologyjunction.com/stemcell_article.htm

1200px-Edinburgh_July_2014_IMG_4384_(14507598508).jpg (1200×900) (wikimedia.org)

for-web-art-new-roadmap-for-cancer-treatment.jpg (1170×680) (weareworldquant.com)