ELECTRONIC AUTOMATIC TEMPERATURE CONTROL OF CRYOPRESERVATION FOR ARTIFICIAL INSEMINATION (PROTOTYPE)

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To my Father, Mother

&

Teachers

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ABSTRACT

The scientist and researchers are facing problems in genetically stabilizing living cells. The living cells can be stabilized by keeping them in cryogenic temperatures. Stabilizing cells at cryogenic temperatures is called cryopreservation, an applied aspect of cryobiology, or the study of life at low temperatures. Advances in cryopreservation technology have led to methods that allow low-temperature maintenance of a variety of cell types. Techniques are available for the preservation of microorganisms, isolated tissue cells, small multi cellular organisms, and even more complex organisms such as embryos. The freezing process involves complex phenomena which, even after decades of research, are not fully understood. The aim of this project to make prototype of a cryopreservation system, so, it can preserve the sperm or any other biological material. For the aim of cryopreservation, a circuit board with a microcontroller, thermocouple controller, and a voltage driver for valve are constructed. Two sensors are used, a K-types thermocouple and Water level detector-brick sensor, which send data to the microcontroller. After the microcontroller receives the data, the data of temperature and level are compared to control the valve. The cryopreservation system is modeled in AutoCAD and the temperature control circuit is programmed in MPLAB. An adjustable plastic box which is non-conductive and resistant to heat is used in the fabricated system. It has a metal box inside to reserve sperm tubes. An electrical solenoid valve is used for supplying necessary liquid. The total cryopreservation is activated by sensing physical value by the sensors which are converted by thermocouple amplifier in understandable form for microcontroller. The data are then processed, compared and the valve is then operated accordingly by the microcontroller. The fabricated system is tested at different temperature conditions and it is found that there is very less error. The system functions very effective and is able to control the temperature of the system to preserve the sperm.

ABSTRAK

Para saintis dan penyelidik menghadapi masalah dalam genetik menstabilkan sel-sel hidup. Sel-sel hidup boleh stabil dengan menyimpannya dalam suhu kriogenik. Penstabilan sel-sel pada suhu kriogenik dipanggil krioawetan, satu aspek gunaan cryobiology, atau kajian hidup pada suhu rendah. Kemajuan dalam teknologi krioawetan telah membawa kepada kaedah yang membolehkan penyelenggaraan rendah-suhu pelbagai jenis sel. Teknik yang disediakan untuk pemeliharaan mikroorganisma, sel-sel tisu terpencil, organisma kecil yang berbilang sel, dan organisma yang lebih kompleks seperti embrio. Proses pembekuan melibatkan fenomena yang kompleks tidak difahami sepenuhunya, walaupun beberapa dekad penyelidikan. Tujuan projek ini untuk membuat prototaip sistem krioawetan, jadi, ia boleh memelihara sperma atau apa-apa bahan biologi lain. Bagi tujuan krioawetan, papan litar dengan pengawal mikro, pengawal suhu dan pemandu voltan untuk injap dibina. Dua sensor digunakan, suhu K-jenis dan paras air sensor pengesan-bata, yang menghantar data ke mikropengawal. Selepas mikropengawal menerima data, data suhu dan tahap dibandingkan untuk mengawal injap. Sistem krioawetan adalah model dalam AutoCAD dan litar kawalan suhu diprogramkan dalam MPLAB. Sebuah kotak plastik laras yang bukan konduktif dan tahan haba yang digunakan dalam sistem yang direka. Ia mempunyai kotak logam dalam untuk tiub sperma rizab. Injap solenoid elektrik digunakan untuk membekalkan cecair yang diperlukan. Jumlah krioawetan diaktifkan dengan mengesan nilai fizikal oleh sensor yang diubah oleh penguat suhu dalam bentuk mudah difahami bagi mikropengawal. Data-data ini kemudiannya diproses, dibandingkan serta injap itu dikendalikan sewajarnya oleh pengawal mikro. Sistem fabrikasi diuji pada keadaan suhu yang berbeza dan ia didapati bahawa terdapat ralat yang sangat minima. Fungsi sistem ini sangat efektif dan mampu untuk mengawal suhu sistem untuk memelihara sperma.

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CHAPTER 1

INTRODUCTION

1.1 Back ground:

Artificial insemination (AI) – is one of the most imperative techniques ever devised for the genetic enhancement of farm animals. It is used extensively as a tool for livestock breeding and management programs. AI is a procedure by which sperms are collected from the male, processed, stored and artificially introduced into the female reproductive tract for the purpose of conception.

A male animal produces millions of sperms daily. Theoretically, it can inseminate females regularly and produce several off springs. Artificial insemination is used instead of natural mating for reproduction purposes. This is when a male animal, for example, a bull, is kept with a herd of cows and 'covers' (copulates with) them when they are ready to mate so the bull's semen fertilizes the cow's eggs to produce calves. Fertilization can take place away from the bull and the two animals do not even meet. Although AI (in the form of intrauterine insemination) is not frequently used in human patients, it is the most commonly used method of breeding food production animals in developed countries, with more than 90% pigs and almost the same proportion of dairy cattle bred by this method in the European Union and North America [1].

In the actual procedure used, semen is obtained from a male animal and, after being diluted, is deep-frozen, after which it can be stored for long periods of time without losing its fertility. For use, the semen is thawed and then introduced into the genital tract of a female animal.

Cryobiology is the study of the effects of extremely low temperatures on biological systems, such as cells or organisms. Cryopreservation—an applied aspect

of cryobiology has resulted in methods that permit low temperature maintenance of a diversity of cells.

The objective of cryopreservation is to minimize damage to biological materials, including tissues, mammalian cells, bacteria, fungi, plant cells and viruses, during low temperature freezing and storage. Cryopreservation provides a continuous source of tissues and genetically stable living cells for a variety of purposes, including research and biomedical processes.

A basic principle of cryobiology is that the extent of freezing damage depends on the amount of free water in the system and the ability of that water to crystallize during freezing. Water is the major component of all living cells and must be present for chemical reactions to occur within a cell. During freezing, most of the water changes to ice, and cellular metabolism ceases. However, obtaining reproducible results for more complex tissues, such as heart valves or engineered tissue constructs, or more sensitive cell types, requires an understanding of the major variables involved in tissue cryopreservation [1].

The first successful experiment with artificial insemination in animals was performed by an Italian physiologist Lazzaro Spallanzani in 1780. He developed a technique for artificial insemination in dogs. This approach was distinguished commercially in 1930 in Russia and the subsequent development of methods for the cryopreservation was developed.

Cryopreservation is a process where cells or whole tissues are preserved by cooling to sub-zero temperatures, typically 77 K (= −196 °C, the boiling point of liquid nitrogen) of semen led to the wide spread use of AI in animals[4].

There are many advantages to artificial insemination (AI) in domesticated and zoo animals. They are smaller chance of injury to either partner during the mating process, X Preface less stress to the female, who is often the one transported to and from the home of the male, the system of reproduction is perfect, including artificial insemination. Examples of wild animals that have been successfully impregnated through artificial insemination include big cats (e.g., the tiger, the puma, the cheetah, and the clouded leopard etc.) [9].

1.2 **Problem Statement**

Cryopreservation is a process where cells or whole tissues are preserved by cooling to sub-zero temperatures, typically 77 K (= −196 °C, the boiling point of liquid nitrogen). The control strategy of temperature until now is done manually and the maintenance is done by rule of thumb or experience. Hence automatic temperature control system is required which can give significant compensation in production of animal or person. It will also be economical and does not create losses to farm industries.

The motivation for this project is to have Cryopreservation which can control temperature automatically and can preserve sperm for a long time. The transportation will also be easy which can minimize the risk of damage to the semen and can increase the farm production.

1.3 National and international status about AI

As per Food and Agriculture Organization of United Nations (FAO), AI coverage in developed countries in 1995 for dairy cattle was 64% and 7.8% for beef cattle compared to developed countries, AI application in Asian countries is very low. In Chi na it was 12.6% 1993 and in Indonesia it was 17.4% in 2000 and 23.1% in 2002. For Malaysian scenario, the AI coverage was 4.3% in 2002 and it remained same till 2009, which is quite similar for several years and is extremely low rate measure compared to Indonesia [2].

Table 1.0: Data of AI cases in different years in Malaysia [2].

Year	1981	1982	1983	1984	1985	1986	1987	1988
Cases	13,357	15,466	19,282	20,532	19,930	22,072	19,533	22,864
Year	1989	1990	1991	1992	1993	1994	1995	1996
Cases	22,787	25,864	19,145	23,372	24,862	22,393	15,972	22,325
Year	1997	1998	1999	2000	2001	2002	2003	2004
Cases	19,243	16,650	15,845	17,527	16,208	18,276	16,867	15,810
Year	2005	2006	2008	2008	2009			
Cases	15,676	18,369	19,021	19,752	18,599			

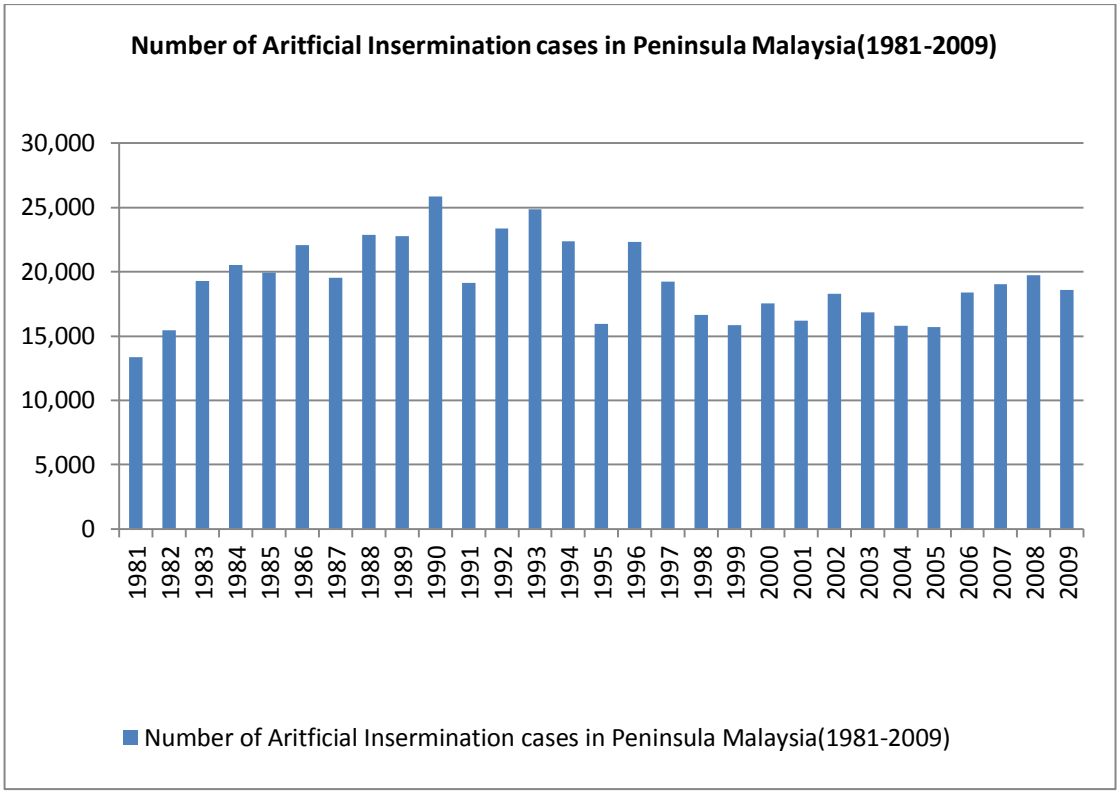

Fig 1.1: Graphical representation of AI in Malaysia [2]

1.4 Objectives:

This project aims to achieve the following objectives;

To design and fabricate an automatic temperature control cryopreservation device for sperm storage which can maintain the quality of sperm and increase the animal production with less labor cost.

1.5 Scope of the project:

The Project is developed for automatic control of the temperature of the cryopreservation system. The following are the scope of the project;

The artificial insemination and cryopreservation system is to be studied to appreciate the significance of cryopreservation system in AI. This study will identify the quality assurance for sperm or any other similar biological material and the importance of freezing rate for controlling the temperature. Design a Cryopreservation tank using a computer aided design tool (CAD). Auto CAD 2010 is to be used to model the plastic cool box with metal box inside which is a good conductor of temperature.

Design a temperature control circuit for controlling the system with glass braid K type with range of -270 to +1372 C thermocouple sensor, a thermocouple amplifier SN-6675 for amplifying the weak voltage and to send the amplified signals to the microcontroller.

Water detects Brick sensor is to be used which will detect the water level.
An electrical solenoid valve AQT15S made up by plastic material and operated by DC12V or DC24 V or 220 AC is to be used. This valve in the system is to control the flow of liquid or water inside the tank. The one end of the valve attached to preserver and other end is attached to the tank. It is driven by a motor driver MD10C. SK40C model microcontroller system with PIC16F877A and PIC Kit 2

V2.61 is to be used to control the entire system. MPLAB IDE V8.46 is to be used for Programming the microcontroller. It has a display unit to display the temperature and hence monitoring will be easier. The micro controller programming is to be written in a high level language ‘C’.

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