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EFFECT OF VITRIFICATION ON SPERMATOZOA QUALITY IN BULL SEMEN

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EFFECT OF VITRIFICATION ON SPERMATOZOA QUALITY IN BULL SEMEN

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CERTIFICATION

It is hereby certified that we have read this project paper entitled "Effect of Vitrification on Spermatozoa Quality in Bull Semen", by Lee Lian Yu and in our opinion it is satisfactory in terms of scope, quality, and presentation as partial fulfillment of the requirement for the course VPD 4999 – Project.

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DEDICATION

This project paper is dedicated

To my family,

Father

Mother

Brothers

And to all my teachers who have committed themselves towards the noble cause of education.

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It is with deepest appreciation and gratitude that I thank all those who have made this project paper a reality.

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Lastly, I wish to extend my sincere thanks to anyone that assisted me directly and indirectly in this project and my most heartfelt gratitude to my family; father, mother, aunties, brothers and my lab mate for their endless love and support throughout my studies.

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LIST OF ABBREVIATIONS

%	Percentage
<	Less than
>	More than
℃	Degree Celsius
kg	Kilogram
g	Gram
L	Liter
mL	Milliliter
M	Molar
hr	Hours
min	Minutes
s	Seconds
cm	Centimeter
x	Times
EE	Electro-ejaculation
TEYG	Tris-egg yolk glycerol extender
HS	Holding solution
VS	Vitrification solution
DMSO	Dimethyl sulfoxide
EG	Ethylene glycol
LN_2	Liquid nitrogen
CASA	Computer-assisted sperm analysis

ABSTRAK

Abstrak daripada kertas projek yang dikemukakan kepada Fakulti Perubatan Veterinar untuk memenuhi sebahagian daripada keperluan kursus VPD 4999 – Projek.

KESAN VITRIFIKASI TERHADAP KUALITI SPERMA PADA SEMEN LEMBU

Oleh

Lee Lian Yu

2017

Penyelia: Prof. Dr. Abd Wahid Haron

Kriopreservasi sperma merupakan salah satu pembantuan bioteknologi reproduksi untuk meningkatkan kapasiti reproduksi dalam haiwan ternakan. Kriopreservasi sperma secara konvensional menggunakan teknik pembekuan berperingkat secara perlahan menyebabkan pembentukkan penghabluran ais dan kriokerosakan dengan menghasil kualiti sperma yang kurang memuaskan selepas dinyahbeku. Oleh itu, vitrifikasi diperkenalkan dengan memejal cecair kepada keadaan berkaca tanpa penghabluran dengan cepat dan murah. Kaedah penyejukan ultra-pantas ini memerlukan bahan kriopreservasi yang berkepekatan tinggi yang bakal tosik kepada sperma. Oleh itu, kajian ini dikendalikan untuk mengenal pasti kesan vitrifikasi kepada kualiti semen lembu. Sejumlah lapan (8) sampel semen

lembu dikumpul menggunakan kaedah elektro-ejakulasi. Media berdasarkan Tris digunakan untuk membandingkan dengan media vitrifikasi yang berlainan konsentrasi krioprotectant pada kadar 10% (media vitrifikasi 1; VS-1) dan 20% (VS-2) mengandungi dimetilsulfoksida (DMSO) serta ethilen glikol. Keputusan memaparkan mortaliti yang tinggi serta parameter motiliti yang hampir sifar pada semua sperma vitrifikasi yang dinyahbeku. Akan tetapi, parameter motiliti keseluruhan dan progresif sperma bagi VS-1 pada penilaian awal adalah 22.45% dan 24.87% lebih baik dan ketara secara statistik berbanding dengan media berdasarkan Tris. Kesimpulannya, vitrifikasi berpotensi sebagai alternatif untuk kriopreservasi. Penyelidikan lanjut mengenai teknik vitrifikasi sperma dalam bidang penyejukan dan pemanasan haruslah dikaji.

Kata kunci: lembu, sperma, kriopresevasi, vitrifikasi, elektro-ejakulasi

ABSTRACT

Abstract of the project paper presented to the Faculty of Veterinary Medicine in partial requirement for the course VPD 4999 – Project.

EFFECT OF VITRIFICATION ON SPERMATOZOA QUALITY IN BULL SEMEN

by

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2017

Supervisor: Prof. Dr. Abd Wahid Haron

Cryopreservation of spermatozoa is part of the assisted reproductive biotechnology to enhance reproductive capacity in livestock. Conventional cryopreservation applies slow-gradual freezing method permitted ice crystallization and causes cryodamage resulting in poor post-thawed semen quality. Hence, vitrification is introduced by solidifying the solution into glassy state without causing any crystallization in fast and inexpensive manner. This ultra-rapid cooling method requires high concentration of cryoprotectants that potentially toxic the spermatozoa. Therefore, this study was conducted to determine the effect of vitrification on the quality of bull semen. A total of eight (8) bull semen samples were collected using electro-ejaculation. Tris-based extender was compared with vitrification using solution of different concentration of cryoprotectants at 10%

(Vitrification Solution 1; VS-1) and 20% (VS-2) containing dimethyl sulfoxide (DMSO) and ethylene glycol respectively. The result revealed that high mortality and nearly zero motility in all post-warmed vitrified spermatozoa, but the general and progressive motilities parameters in VS-1 at initial evaluation was 22.45% and 24.87% respectively better than Tris-based extender and was statistically significant. In conclusion, vitrification has potential as an alternative for cryopreservation. Therefore, further research on spermatozoa vitrification technique on enhancement in cooling and warming should be conducted and investigated.

Key words: bull, spermatozoa, cryopreservation, vitrification, electro-ejaculation

CHAPTER I

GENERAL INTRODUCTION

Cryopreservation is the preservation of structurally intact living cells using very low temperatures (Wolkers and Oldenhof, 2015) which played an important role in livestock reproduction. Assisted reproductive biotechnology (ARB) has been applied in various species of animalsto enhance reproductive capacity, to improve and preserve livestock genetics, as well as to develop new animal products. Artificial insemination is an example of ARB whereby various studies and development programmes have been conducted to improve the quality of cryopreserved semen. Bovine semen is the least sensitive of all species to freezing damage (Holt, 2000) but the biological effects of conventional cryopreservation is dominated by freezing of water resulting in high concentration of solutes, leading to osmotic shock as well as intracellular and extracellular damage. Hence, cryoprotective agents (CPA) were introduced to increase the total concentration of all solutes in the media and reduce the amount of ice formed at any given temperature (Pegg, 2015). The CPAs that administeredmust be biologically acceptable and able to penetrate into the cells with low toxicity, but the concentration that required varies according to species, cell types and procedures(Pegg, 2015).

The slow freezing technique applied in conventional cryopreservation causes intracellular freezing and extracellular ice formation that potentially damaged the spermatozoa. Thus, interventions have been conducted to avoid ice formation by vitrification. This technique allows production of a glassy state that is defined by the

viscosity reaching a sufficiently high value of 10^{13} poise to act like a solid without causing any crystallization. However, toxicity due to high concentration of CPA that used in vitrification is the major obstacles (Pegg, 2015).

Conventional cryopreservation is rather expensive and time-consuming, even though lesser amount of CPA is required. Formation of ice crystal during slow freezing increases cyrodamage which produced poor to moderate result of viable spermatozoa after thawing. In contrast, vitrification is an inexpensive, ultra-rapid freezing method that preserve cells to sub-zero temperatures whereby fast cooling rate results in solidification of solution into glass-like structure rather than the ice crystals which reduces cryodamages. High CPA concentration is required and it is toxic to the cells, therefore exposure time to perform this technique must be conducted rapidly. However, complete understanding and development of vitrification in bovine spermatozoa is yet to be discovered.

Thus, the objectives of this study are to:

1. To determine the suitability of freezing bull semen by vitrification.
2. To evaluate the quality of vitrified bull semen.

Therefore, the hypothesis of this project is that the quality of vitrified bull semen is better than conventional cryopreservation.

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