



UNIVERSITI PUTRA MALAYSIA

***EFFECTS OF DIFFERENT CONCENTRATION OF
DIMETHYLSULFOXIDE AND GLYCEROL ON MOTILITY,
MORTALITY AND MORPHOLOGY OF CRYOPRESERVED BULL
SEMEN***

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GLYCEROL ON MOTILITY, MORTALITY AND MORPHOLOGY OF
CRYOPRESERVED BULL SEMEN

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It is hereby certified that we have read this project paper entitled “Effects Of Different Concentration Of Dimethyl Sulfoxide And Glycerol On Motility, Mortality And Morphology of Cryopreserved Bull Semen”, by AfiqahBintiZainurin and in our opinion it is satisfactory in terms of scope, quality, and presentation, as partial fulfilment of the requirement for the course VPD 4901 – Project.

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DEDICATION

This thesis is dedicated
To My Teachers and My Parents
For their profound gratitude
And
To My Fiance
Amir Hamzah Bin Ahmad
For his eternal love

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ABSTRAK

Abstrak daripada kertas projek yang dikemukakan kepada Fakulti Perubatan Veterinar untuk memenuhi sebahagian daripada keperluan kursus VPD 4901- Projek.

KESAN PERBEZAAN KEPEKATAN DIMETHYLSULFOXIDE DAN GLISEROL TERHADAP PERGERAKAN, KEMATIAN DAN MORFOLOGI AIR MANI LEMBU YANG DIKRIOAWET

oleh

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2015

Penyelia: Prof. Dr. Abd Wahid Haron

Krioawetan gamet dan embrio secara digunakan secara rutin dan meluas dalam mamalia. Krioawet sperma, oosit dan embrio digunakan untuk peranakan beradas dan pemindahan embrio dalam industry ternakan. Oleh itu, keupayaan untuk meramal kualiti sperma selepas pencairan dan kesuburan dari rutin fungsi sperma akan sangat bermanfaat bagi kejayaan krioawetan. Tujuan kajian ini adalah untuk menilai kesan berbeza kepekatan dimetil sulfoksida (DMSO) dan gliserol pada pergerakan, kematian dan morfologi air mani lembu jantan yang dikrioawet. Dua sampel air mani dari dua ekor lembu jantan telah dikumpul dan dibekukan dalam pencair Tris kuning telur dengan DMSO dan gliserol pada kepekatan yang berbeza-beza iaitu 5%, 10% dan 20%. Data yang diperolehi dibandingkan dengan kumpulan

kawalan yang mengandungi 3% gliserol. Parameter kualiti air mani diuji selepas pencairan ialah peratusan pergerakan sperma progresif, peratusan keseluruhan pergerakan, peratus hidup dan peratus sperma bermorfologi tidak normal. Keputusan menunjukkan gliserol pada kepekatan 5% mempunyai kesan yang paling besar terhadap kemandirian sperma manakala DMSO pada kepekatan 5% memberikan purata kemandirian sperma yang lebih rendah berbanding dengan purata kemandirian sperma kumpulan kawalan kedua-dua untuk hari 1 dan hari 7 selepas pencairan. Kepekatan lain menunjukkan penurunan dalam kemandirian bagi setiap kenaikan kepekatan. Tiada perbezaan yang signifikan pada peratusan purata morfologi tidak normal untuk kedua-dua gliserol dan DMSO ($p > 0.05$). Kesimpulannya, 5% gliserol dalam pencair Tris-kuning telur menunjukkan kemandirian yang baik sebagai bahan krioawetan untuk air mani lembu dikrioawet.

Kata Kunci: Gliserol, DMSO, lembu, air mani, bahan krioawet

ABSTRACT

Abstract from project paper for submission to Faculty of Veterinary Medicine in fulfilment of the requirements for the subject VPD 4901- Project.

EFFECTS OF DIFFERENT CONCENTRATION OF DIMETHYLSULFOXIDE AND GLYCEROL ON MOTILITY, MORTALITY AND MORPHOLOGY OF CRYOPRESERVED BULL SEMEN.

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Cryopreservation of gametes and embryos are routinely and widely applied in the mammalian. Cryopreserved sperm, oocytes and embryos are used for artificial insemination and embryo transfer in the livestock industry. Hence, the ability to predict post-thaw sperm quality and fertility from a routine sperm function assay would be greatly beneficial to the success of cryopreservation. The aim of this study was to evaluate the effects of different concentration of dimethylsulfoxide (DMSO) and glycerol on motility, mortality and morphology of cryopreserved bull semen. Semen samples from two bulls were collected twice from each bull and frozen in egg-yolk Tris extender with variable DMSO and glycerol concentrations of 5%, 10% and 20%. The result was compared with control group which consist of 3% glycerol. Semen quality parameters assessed after thawing were sperm percentage progressive, percentage

total motile, percentage live and percentage abnormal morphology of sperms. Results indicated that 5% glycerol had the highest effect on survivability of the sperm while 5% DMSO had lower survivability compared to the control group both for day 1 and day 7 post thawing. Other concentration shows decreasing in survivability as the concentrations increased. There is no significant difference on mean percentage of abnormal morphology for both glycerol and DMSO ($p > 0.05$). In conclusion 5% glycerol in egg-yolk Tris showed better survivability of cryopreserved bull semen.

Keywords: Glycerol, DMSO, bull, semen, cryoprotectant

CHAPTER 1

GENERAL INTRODUCTION

Artificial insemination is widely used to produce the good and valuable genetics. It is the first animal biotechnology advancement that has made a large contribution to the genetic improvement, particularly in dairy bulls which single ejaculate from males can be used for inseminations of many females (Büyükleblebici *et al.*, 2014). One of the most important processes prior to artificial insemination is semen cryopreservation. It has been indicated that there are many benefits resulting from the process of semen cryopreservation such as increase breeding efficiency, increase ability to access superior genetics for a fraction of the price of buying a bull and extremely important for preservation of superior genes from valuable animals (Lemma, 2011). Cryoprotectant is the term to describe as any additive which can be provided to the cells before freezing and gives a higher post-thaw survival which cannot be obtained in its absence (B.Fuller, 2004).

According to Büyükleblebici *et al.*(2014), the successful of cryopreservation is not depend only on preserving the viability of the spermatozoa but also on maintaining their metabolic functions. Cryoprotactant was added to extender to protect the sperm from damage during freezing process. The amount and type of cryoprotectant also influence the viability of the spermatozoa. Therefore, the suitable cryoprotectant and extender are important factors for successful semen cryopreservation (Dorjiet *et al.*, 2014).

A cryoprotectant concentration of about 5% to 15% is usually all that is required to permit survival of a substantial fraction of isolated cells after freezing and thawing from liquid nitrogen temperature (Jennifer, 2007). Jennifer (2007) also mentioned that the best and most commonly used cryoprotectants are a class of cryoprotectants called penetrating cryoprotectants which are small molecules that easily penetrate cell membranes and prevent excessive dehydration of cells during the freezing process.

The property of glycerol that can reduce the mechanical damage to spermatozoa during the freezing process makes it as most widely used cryoprotectant for bull semen. Conversely, according to Martins-Bessa *et al.* (2006), glycerol can induce alterations in the viscosity and organization of the sperm cytoplasm and in stability and permeability of the plasma membrane through disruption of protein and phospholipid structural organization.

Dimethyl sulfoxide (DMSO) was used in the first human cryopreservation protocol to ease the freezing process. According to Gurtovenko & Anwar (2007), DMSO is observed to exhibit distinct modes of action in different concentration ranges which at low concentrations, DMSO induces membrane thinning and increases fluidity of the membrane's hydrophobic core and at higher concentrations, DMSO induces transient water pores into the membrane.

Therefore, the objectives of this study were:

1. To determine the effects of different concentrations of DMSO and glycerol on the survival rate of cryopreserved bull semen

2. To evaluate the mortality, motility and morphology of cryopreserved bull semen post thawing on day 1 and day 7 DMSO and glycerol as cryoprotectants.



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