EFFECT OF DIFFERENT THAWING TEMPERATURE AND TIME ON THE VIABILITY OF CRYOPRESERVED BOVINE SPERMATOZOA

INSTITUT PENGURUSAN PENYELIDIKAN

UNIVERSITI TEKNOLOGI MARA

40450 SHAH ALAM, SELANGOR

MALAYSIA

BY :

FARIDA ZURAINA HJ MOHD YUSOF

SYARIFAH FAEZAH SYED MOHAMAD

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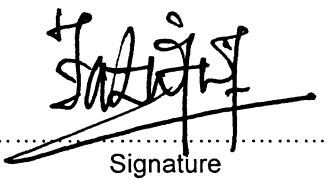
PROJECT TEAM MEMBERS

ASSOC. PROF. DR. FARIDA ZURAINA HJ MOHD YUSOF
Project Leader



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SYARIFAH FAEZAH SYED MOHAMAD
Project Member

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Abstract

Cryopreservation is a technique in order to preserve cell for long time storage. It is widely used in agricultural field to store male gametes in the use of liquid nitrogen. In this study, three adult bulls breed Piedmontase were used. Semen collected from these bulls was then grouped into two; treatment group was subjected to magnetic activated cell sorting system (MACS) and being cryopreserved, whilst the control group was directly cryopreserved without MACS. Sperm were incubated in Annexin V before placed in the column attached to the magnetic stand. Semen analysis was done by using the Computer Assisted Sperm Analysis software (CASA). Parameters included are sperm velocity (VAP, VCL, VSL; μm/s) and sperm progression (WOB, LIN, STR; %). For sperm viability it was determined by eosin-nigrosin staining technique. Effects of different thawing temperature and time were analysed by using iQ™SYBR® Green assay for qPCR with GAPDH as reference gene. Cryocapacitation like damage was then identified by the chlorotetracycline/Hoechst staining assay under blue-violet illumination of fluorescent microscopy (excitation: 400-440nm, emission: 470nm). All statistical data were analysed using SPSS version 14; p-value was set at 0.05 as significant level. Findings revealed that thawing at 37°C for 13 seconds for MACS processed sample was superior compared to other thawing procedures. At 37°C, it produced significant data of VCL μm/s, expression of HSP70 gene and CTC/Hoechst assay with $p<0.05$. As a conclusion, usage of MACS preceding cryopreservation can enhance cell survival by thawing at 37°C for 30 seconds.

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