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CHARACTERISTICS OF FROZEN-THAWED SEMEN OF DIFFERENT CATTLE BREEDS, STORAGE TIME, PACKAGING AND SOURCE OF PRODUCTION

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CERTIFICATION

It is hereby I certified that we have read this paper project entitled "Characteristics of frozen-thawed sperm of different breeds, storage time, packaging and source of production", by Nor LiyanabintiMohdDzin and our opinion it is satisfactory in terms of scope, quality, and presentation as partial fulfilment of the requirement for the

course VPD 49999 – Project.

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DEDICATIONS

In the name of Allah, the Beneficient, and the Merciful.

This thesis is dedicated especially to my parents

andmy family.

Thank you for your endless love and support.

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ABSTRAK

Abstrak kertas projek yang dikemukakan kepada Fakulti Perubatan Veterinar untuk memenuhi

sebahagian daripada keperluan kursus VPD4999- Projek

CIRI SPERMA SEJUK BEKU-CAIRDARIPADA BAKA LEMBU, MASA DAN BEKAS PENYIMPANAN, DAN SUMBER PENGHASILAN YANG BERBEZA

Nor Liyana binti Mohd Dzin

2016

Penyelia: Assoc. Prof. Dr.RosninaYusoff

Penyelia bersama: Prof.Dr. Mohamed Ariff Omar

Tujuan kajian ini dijalankan adalah untuk membandingkan kemotilan umum dan progresif, kebolehidupan,dan ciri keabnormalan sperma sejuk beku-cair dari baka lembu, masa dan bekas penyimpanan, dan sumber penghasilan yang berbeza.Enam puluh enam sampel semen sejuk beku daripada sebelas baka lembu, disimpan di dalam ampul kaca atau straw yang dihasilkan dari dalam atau luar Negara dari tahun 1975 sehingga 2010 telah diuji. Keputusan analisis menunjukkan masa dan bekas penyimpanan, dan baka lembu memberi kesan yang bermakna terhadap kemotilan umum dan progresif semen sejuk beku-cair. Manakala, sumber penghasilan memberi kesan yang bermakna terhadap kemotilan progresif, kebolehidupan semen sejuk beku-cair tetapi tidak terhadap motilitiumum.Masa penyimpanan, baka lembu dan sumber penghasilan

yang berbeza memberi kesan yang bermakna terhadap kebolehidupan dan ciri keabnormalan semen sejukbeku-cair.Kesimpulannya, lembu baka Holstein mempunyai kualiti semen yang terbaik di dalam kajian ini. Semen sejuk beku-cair yang dihasilkan pada tahun 1999 adalah yang terbaik berbanding tahun yang lain. Semen sejuk beku-cair yang disimpan di dalam straw mempunyai kadar motility umum lebih tinggi berbanding ampul kaca. Manakala, semen sejuk beku-cair yang disimpan di dalam ampul kaca mempunyai kadar motility lebih tinggi berbanding straw. Semen sejuk beku tempatan telah diuji lebih berkualiti berbanding semen yang diimport.

Kata Kunci :kemotilan, kebolehidupan, morfologi, sejuk beku-cair, mengkrioawet

ABSTRACT

An abstract of the project paper presented to the Faculty of Veterinary Medicine in partial fulfilment of requirement for the course VPD 4999 – Project

CHARACTERISTICS OF FROZEN-THAWED SPERM OF DIFFERENT CATTLE BREEDS, STORAGE TIME, PACKAGING AND SOURCE OF PRODUCTION

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2016

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The aim of this study is to compare the general and progressive motility, viability, and abnormality characteristics of frozen-thawed semen of different cattle breeds, duration of storage, packaging, and source of production. Sixty six semen samples from eleven breeds packed either in glass ampules or straws that were produced locally or were imported between 1975 and 2010 were evaluated for the above characteristics. Results showed that general motility was significantly different in the different storage duration, breeds and packaging, but not significantly different in the source of production. Progressive motility of thawed sperm was also significantly different in different storage duration, breeds, packaging, and source of

production. Viability and abnormality of thawed sperm were significantly different in different storage duration, breeds, and source of production but not significantly different in packaging. Based on the results, it can be concluded that Holstein frozenthawed semen was the best quality among other breeds. Frozen-thawed semen produced in 1999 was the best quality among the other years. Between packaging, straws have higher general motility compared to glass ampules.While, glass ampules have higher progressive motility compared to straws. Locally produced frozenthawed semen was examined to be better compared with imported semen

Keywords: motility, viability, morphology, frozen-thawed, cryopreserved

1.0 INTRODUCTION

Recent local beef and dairy production can only fulfil 25.67% and 12.93% of country self-sufficiency (DVS, 2013). Beef and dairy sectors of the livestock industry, however, are still 84.43% and 87.7% in shortage, although the total population of ruminant has increased over the last decade. By 2020, Malaysian government has targeted to raise the self-sufficiency level of beef to 32.7% (MOA, 2015), which can be translated as slaughter of more than 450,000 heads of cattle anually.Moreover, the demand for livestock products as a source of high quality protein is expected to continue to rise with the increase in population and per capita income, consistent with the overall rapid development of the country (Talukder, 2002).

An approach to the development of cattle industry in Malaysia is by increasing the population and productivity of cattle livestock. An artificial insemination (AI) is one good tool. AI can improve the genetics of dairy cattle in the minimum possible time (Malik, 2015). It was the first assisted reproductive technology to be applied commercially for the genetic improvement of animal in the mid-1900s until now (Peter, 2007). Artificial insemination (AI) with cryopreserved semen is the predominant method used in cattle reproduction around the world. According to Mustafa (1974), in Malaysia the first usage of AI using deep frozen semen from ampoules was in 1963. The initial purpose of using AI was the obtain crossbreds which can produce higher milk and beef production, the animal also less susceptible to disease as compared to the imported pure breeds (Raymond, 2010). The success of AI program depends on several factors. The factors include high quality of semen, genetics, physiology, nutrition and management of cows (Walsh *et al.*, 2011).Among the aforementioned, factors for semen quality is considered most critical especially frozen thawed semen mainly biophysical and biochemical characteristics of sperm (Medeiros *et al.*, 2002). Furthermore, Hayashi and Ishobe (2005) reporteded that frozen thawed semen parameters such as viability, motility, and abnormality are crucial factors for AI to be successful. High viability, motility and abnormality of frozen-thawed spermatozoa are significant factor because the relationship between the post-thawing sperm viability and the subsequent conception rate has been reported (Correa JR, 1997).

Semen cryopreservation halts metabolic processes of spermatozoa and allowing indefinite storage without a significant loss of fertility(Lemma, 2011). It is important that spermatozoa are cryopreserved for a longer period without damaging their fertilizing ability. Frozen semen can be stored indefinitely to facilitate their use at any time, depending on the oestrous cycle of females. However, damage of sperm during freezing and thawing can lead to low sperm motility and consequently, fertility.

To maximally utilize the genetics of desired sires on a commercial basis, attempts are made to package a minimal number of spermatozoa per insemination unit without compromising fertility (Foote and Parks, 1993; Shannon and Vishwanath, 1995). Later, the introduction of plastic straws has resulted in the use of smaller volumes (0.25ml and 0.5ml) of frozen semen so that more females can be inseminated from a single ejaculate.

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Nowadays, straws are generally popular as they are very convenient and easy to use in artificial inseminator. As different methods of packing have been used, the question is whether the means of packing has any effect on the success rate of cryopreservation. In this regard, different authors have compared spermatozoa stored in different packages (Heitland et al, 1996; Kneissl 1993; Park et al, 1995). However, there are some discrepancies in the study and was not fully explained.

There are about twelve breeds of beef and dairy sires cryopreserved in Theriogenology and Cytogenetics Unit, Faculty of Veterinary Medicine, UPM. The semen quality among these breeds in terms of sperm survivability in frozen and after thawing are unknown as there are no study done. Frozen thawed semen quality information is crucial for AI to be successfull.

The application of frozen-thawed semen technology is currently increasing worldwide including Malaysia. Theriogenology and Cytogenetics Unit, Faculty of Veterinary Medicine, UPM is one of the centre that having expertise in the production of high quality cryopreserved semen. The centre has been producing good quality of cryopreserved sperm for many years. Numbers of studies and developments have been done regarding the cryopreservation technique and the effects of sperm quality and fertility. The quality of cryopreserve semen is thought to be as good as imported. However, the quality to compare the cryopreserve semen between local and imported production has not been tested.

Thus, this project was conducted to depict the effects of storage duration and packaging materials on sperm parameters such as motility, viability and abnormality.

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In addition, seminal characteristics of frozen-thawed semen from different breeds of sires and source of production are also investigated.



8.0 REFERENCES

Albert DB (2007). Evaluation of potential Breeding Soundness of the bull.In R.S. Younquist& W.R. Threlfall (2). , Large animal theriogenology(228-239). United States of America: Sounder"s Elsevier.

Andrabi SMH, 2007. Fundamental principles of cryopreservation of BosTaurus and Bosindicus bull spermatozoa. Mini review. Int. J. Agri. and Biol.; 9:367-369

Arav A, Yavin S, Zeron Y, Natan D, Dekel Y, Gacitua H (2002) New trends in gamete"s cryopreservation. Mol Cell Endocrinol 187:77–81. doi:10.1016/S0303-7207(01)00700-6

Barbas, J. P., & Mascarenhas, R. D. (2009).Cryopreservation of domestic animal sperm cells. *Cell and tissue banking*, *10*(1), 49-62.

Bratton, R.W., Foote, R.H. and Henderson ,C.R.(1954). The relationship between fertility and the number of spermatozoa inseminated. J. Dairy Sci. 37, 1353-1356.

Check ML, Check JH, Long R: Detrimental effects of cryopreservation on structural and functional integrity of the sperm membrane. Arch Androl 1991;27:155–160

Chenoweth, P. J. (2005). Genetic sperm defects. Theriogenology, 64(3), 457-468.

Clulow JR., L.J. Mansfield, LHA. Morris, G. Evans, and WMC.Maxwell, 2008.A comparison between freezing methods for the cryopreservation of stallion spermatozoa, Anim. Reprod. Sci. 108:298-308

- Cooter PZ, HA Goolsby, and SD Prien, 2005.Preliminary evaluation of a unique freezing technology for bovine spermatozoa cryopreservation.Reprod. Dom. Animal; 40:98-99
- Correa JR. Relationships among frozen-thawed sperm characteristics assessed via the routine semen analysis, sperm functional tests and fertility of bulls in an artificial insemination program. Theriogenology 1997; 48; 721-731.
- Curry, M. R. (2000). Cryopreservation of semen from domestic livestock.*Reviews of reproduction*, 5(1), 46-52.
- DVS. 2015. Livestock Statistics 2014. Department of Veterinary Services.
- Eriksson, G., Namkoong, G., & Roberds, J. H. (1993). Dynamic gene conservation for uncertain futures. *Forest Ecology and Management*, 62(1), 15-37.
- Foote RH and JE Parks, 1993. Factors affecting preservation and fertility of bull semen: a brief review. Reprod. Fertil. Dev.; 5:665-73
- Hafez ESE. Preservation and cryopreservation of gametes and embryos: Reproduction in farm animals. 5ed. Philadelphia: Lea an Febiger;1987, p. 591.
- Hammerstedt RH, Graham JK, Nolan JP. Cryopreservation of mammalian sperm: what we ask them to survive.JAndrol 1990; 11; 73-88.
- Hawk HW(1983) Sperm survival and transport in the female reproductive tract Journal of Dairy Science 662645–2660
- Hayasi I, Isobe N. Characteristics of cryopreserved spermatozoa from a Holstein-Friesian bull thawed at different temperature. J Interl Develop & Cooperation 2005; 12 (1):107-110.
- Heitland AV, DJ Jasko, JK Graham, EL Squires, RP Amann, and BW Pickett, 1995.Motility and fertility of stallion spermatozoa cooled and frozen in a modified skim milk extender containing egg yolk and liposome.Biol. Reprod.Mono.; 1:753-759
- Herman, H.A. and Madden, F.W.(1963). The Artificial Insemination of dairy and beef cattle. A hand book of laboratory manual, Freeman and Company, San Francisco, USA., pp. 579-610
- Holt, W. V. (2000). Fundamental aspects of sperm cryobiology: the importance of species and individual differences. *Theriogenology*, *53*(1), 47-58.

- J.V. Braun, The role of livestock production for a growing world population, Lohmann Information, 45, 2010, 3-6
- Lemma, A. (2011). *Effect of cryopreservation on sperm quality and fertility*.INTECH Open Access Publisher.
- Liebermann, J., Nawroth, F., Isachenko, V., Isachenko, E., Rahimi, G., & Tucker, M. J. (2002).Potential importance of vitrification in reproductive medicine. *Biology of reproduction*, 67(6), 1671-1680.
- Malik, A., Laily, M., &Zakir, M. I. (2015). Effects of long term storage of semen in liquid nitrogen on the viability, motility and abnormality of frozen thawed Frisian Holstein bull spermatozoa. *Asian Pacific Journal of Reproduction*, 4(1), 22-25.
- Mattner PE, Entwistle KW and Martin ICA(1969) Passage, survival and fertility of deep-frozen ram semen in the genital tract of the ewe Australian Journal of Biological Science 22181–187
- Medeiros CMO, Forell F, Oliveira ATD, Rodrigues JL. Current status of sperm cryopreservation: why isn't it better? Theriogenology 2002; 57:327-344.
- Menon, A. G., Barkema, H. W., Wilde, R., Kastelic, J. P., &Thundathil, J. C. (2011). Associations between sperm abnormalities, breed, age, and scrotal circumference in beef bulls. Canadian Journal of Veterinary Research, 75(4), 241-247
- MOA.2015. www.moa.gov.my/documents.
- Mustafa A.B (1974). The development of the cattle Artificial Insemination Service in Peninsula Malaysia.Background Paper No. 2, Animal division, Ministry of agriculture and fisheries, Malaysia.
- Nogueira D, Bourgain C, Verheyen G, Van Steirtegham AC. Light and electron microscopic analysis of human testicular spermatozoa and spermatids from frozen and thawed testicular biopsies. Hum Reprod 1999;14:2041–2049
- Purdy PH (2006) A review on goat sperm cryopreservation. Small Rum Res 6:215–225 Pursel VG, Johnson LA (1975)
- Raymond LN (2007). Techniques for artificial insemination of cattle with frozenthawed semen. In R.S. Younquist& W.R. Threlfall (2). , Large animal theriogenology(253-257). United States of America: Sounder's Elsevier.
- Shamsuddin M, Larsson B (1993) In vitro development of bovine embryos after fertilization using semen from different donors. ReprodDomestAnim 28:77– 84

- Shannon P, and R Vishwanath, 1995. The effect of optimal and suboptimal concentrations of sperm on the fertility of fresh and frozen bovine semen and a theoretical model to explain the fertility differences. Anim. Reprod. Sci.; 39:1-10.
- Špaleková, E., Kulíková, B., BALÁŽI, A., Makarevich, A., &Chrenek, P. (2015).Post-thaw characteristics of pinzgau bull semen following long-term and short-term storage. *Slovak Journal of Animal Science*, *48*(3), 97-102.
- Talukder, M. A., &Talukder, M. A. I. (2002). Characterisation of Productivity Traits of Sahiwal-Friesian Breed Groups (Doctoral dissertation, Universiti Putra Malaysia).
- T.L.N Rao and A.R Rao, Fertility and its relationship with semen characteristics in Crossbred bulls, Indian Vet.J. ,56 ,1979 : 33-36
- Walsh SW, Williams EJ, Evans AC (2011) A review of the causes of poor fertility in high milk producing dairy cows. AnimReprodSci 123: 127-138.
- Watson PF. Recent developments and concepts in the cryopreservation of spermatozoa and the assessment of their postthawingfunction.ReprodFertilDev 1995; 7: 871-891.
- Yoshida, M. (2000). Conservation of sperms: current status and new trends. *Animal Reproduction Science*, 60, 349-355