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COMPARISON OF DIFFERENT CYTOLOGIC STAINING TECHNIQUES ON BOER GOAT SPERMATOZOA MORPHOLOGY AND MORPHOMETRY

SULIZA BT ABD WAHAB

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COMPARISON OF DIFFERENT CYTOLOGIC STAINING TECHNIQUES ON BOER GOAT SPERMATOZOA MORPHOLOGY AND MORPHOMETRY

SULIZA BT ABD WAHAB

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CERTIFICATION

It is hereby certified that we have read this project paper entitled "Comparison of Different Cytologic Staining Techniques on Boer Goat Spermatozoa Morphology and Morphometry", by SulizaBtAbdWahab and in our opinion it is satisfactory in terms of scope, quality, and presentation as partial fulfilment of the requirement for the course VPD 4999 – Project

DR INTAN SHAMEHA BINTI ABDUL RAZAK

DVM (UPM), Ph.D. (UPM)

Senior lecturer

Faculty of Veterinary Medicine

Universiti Putra Malaysia

(Supervisor)

DR MARK HIEW WEN HAN

DVM (UPM), Ph.D. (Purdue)

Lecturer

Faculty of Veterinary Medicine

Universiti Putra Malaysia

(Co-Supervisor)

PROF.DR ABD WAHID HARON

DVM (UPM),Ph.D. (Dublin)

Senior lecturer

Faculty of Veterinary Medicine

Universiti Putra Malaysia

(Co-Supervisor)

DEDICATIONS

Praised to Allah S.W.T., I have completed this project and this project paper is dedicated to

My family,

Father

Mother

Brothers

Friends

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

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LIST OF ABBREVIATIONS

AI	Artificial insemination
^{0}C	Celcius
CASA	Computer-Assisted Sperm Analysis
cm	centimeter
DPX	dibutylphathalate xylene
DQ	Diff-Quik
EN	Eosin-Nigrosin
et al.	and others
fig.	figure
g	gram
HE	Hematoxylin-eosin
hr	hour
mg	milligram
min	minute
ml	milliliter
µl	microliter
NS	normal saline
Obj.	objective
RD	Rapidiff
s	seconds

SE	standard error
SPB	Sorenson Phosphate Buffer
temp	temperature
μm	micrometer
kV	kilovolt
wt	weight

ABSTRACT

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

COMPARISON OF DIFFERENT CYTOLOGIC STAINING TECHNIQUES ON BOER GOAT SPERMATOZOA MORPHOLOGY AND MORPHOMETRY

By

SulizaBtAbdWahab

2016

Supervisor: Dr.IntanShamehabintiAbdul Razak

Co-supervisor:

Dr. Mark Hiew Wen Han

Prof. Dr.Abd Wahid Haron

Sperm morphology is the most reliable parameter for predicting fertility whereas sperm morphometry value as an indicator of reproductive capacity in males. Visual observation has led to widely varying results due to numerous factors such as the use of different staining procedures and lack of standardization of staining techniques. Thus, this study was conducted to compare different cytology staining techniques and to identify the best staining methods for better semen evaluation. Seven semen samples were

collected using a standardized electro ejaculator from Boer bucks at TPU UPM (n = 4), and Labu farms (n = 3). Each sample was primarily assessed using routine semen evaluation protocols and processed accordingly. The semen smears were stained using different stains namely Eosin-Nigrosin (EN), Giemsa (G), Diff-Quik (DQ) and Hematoxylin-Eosin (HE). The slides were examined and morphometric measurements of 50 randomly selected sperm in each stained slides were performed and compared. Morphometric analysis revealed the smallest values recorded in HE for measurements of the head. Results showed there are significant difference ($p<0.05$) of all the morphometric parameters between EN and HE and; DQ and HE. Higher values of head width and length recorded in all stains except HE. It was concluded that there were alteration of measurement in buck sperm morphology with different staining method used.

Keywords: spermatozoa, staining, morphology, morphometry, Boer, buck

ABSTRAK

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

PERBANDINGAN TEKNIK PEWARNAAN SITOLOGI YANG BERBEZA KE ATAS MORFOLOGI DAN MORFOMETRI SPERMATOZOA KAMBING BOER

Oleh

Suliza Bt Abd Wahab

2016

Penyelia: Dr Intan Shameha binti Abdul Razak

PembantuPenyelia:

Dr Mark Hiew Wen Han

Prof. Dr.Abd Wahid Haron

Morfologis perma adalah parameter yang dipercayai bagi meramalkan kesuburan, manakala nilai morfor metris perma digunakan sebagai penunjuk kapasiti pembiakan haiwan jantan.Pemerhatian secara visual secara umumnya telah membawa kepada keputusan yang berbeza-beza kerana pelbagai factor seperti penggunaan prosedur pewarnaan yang berlainan dan kekurangan penyeragaman teknik pewarnaan.Oleh itu, kajian ini dijalankan untuk membandingkan teknik pewarnaan sitologi yang berbeza dan untuk mengenalpasti kaedah pewarnaan yang terbaik bagi penilaian air mani yang lebih baik. Tujuhs ampel air mani dikumpul menggunakan elektro ejakulator daripada kambing jantan Boer di TPU, UPM

(n = 4), dan lading Labu (n = 3). Umumnya, setiap sampel dinilai menggunakan protocol rutin penilaian air mani dan seterusnya diproses. Smear air mani nipis disediakan dan diwarnakan menggunakan teknik pewarnaan yang berbeza yang melibatkan; Eosin-Nigrosin (EN), Giemsa (G), Diff-Quik (DQ) dan Hematoksilin-eosin (HE). Slaid diperiksa dan ukuran morfometri dijalankan keatas 50 sperma yang dipilih secara rawak dalam setiap slaid berwarna dan dibandingkan.Analisis morfometri menunjukkan nilai terendah direkodkan pada HE bagi ukuran kepala.Hasil kajian menunjukkan terdapat perbezaan yang signifikan ($p < 0.05$) bagi semua parameter morfometri antara EN dan HE dan; DQ dan HE.Nilai lebih tinggi bagi lebar kepala dan panjang kepala telah dicatatkan pada semua pewarnaan kecuali HE.Kesimpulannya, terdapat perubahan dalam ukuran dalam morfologis perma kambing dengan kaedah pewarnaan yang berbeza digunakan.

Kata Kunci: spermatozoa, pewarnaan, morfologi, morfometri, Boer, kambing jantan

1.0 Introduction

1.1 Semen Evaluation and Analysis

When choosing a male for breeding, it is important to assess its potential fertility by undertaking clinical and laboratory examinations. Reproduction of the male is closely related to semen quality and normal sperm structure. Therefore, semen analysis is crucial and it comprises of multi-staged process.The semen analysis routinely includes an immediate assessment of volume, appearance sperm concentration and motility, as well as later determination of sperm morphology and the presence of foreign cells. Once screened for normality, ejaculates are assessed for sperm concentration and sperm motility. The morphological evaluation, which is increasingly expanded to include detailed morphometry of the spermatozoon and is considered as a useful tool in the clinical diagnosis of sub fertile animals. Motility assessment can easily be evaluated with a computer-assisted sperm analysis (CASA) instrument. However, the CASA instruments are not in widespread commercial use due to its need for proper programming with species-specific settings, and also the validation requirement.

According to Villaverde*et al*. (2008), the goal for sperm staining technique is to ease the visualization of the cells and provide a better identification of the abnormalities through light microscopy. In the laboratory practice, there are varieties of slide-staining methods used during morphological evaluation of semen to predict male fertility. However, the common encountered problem during evaluation of the morphology and morphometry of sperm is the lack of standardization of staining techniques. Comparison among techniques isessential in order to identify the most

suitable for the species function, because sperms are highly specialized cells operating at a microscopic level in a complex environment (Gage, 1998). Therefore, the method of staining and evaluating semen can significantly affect the results of morphometric measurements.

1.2 Importance of Semen Quality Assessment

Fertility of sperm is the ultimate test of sperm quality. Eventhough fertility cannot be accurately predicted, the deviation of the sperm morphology from the norm is usually a good predictor of low fertility. According to Nuti(2013), theobjective of semen quality evaluationis to identify those samples that have traits pointing to the probability of below normal fertility and therefore discard them, as an extension is to remove the males that continuously produce poor quality semen.

Thus, this study is conductedto compare the simple and practical staining techniques, whichonly using simple equipment, such as a bright field microscope. The main objectives of this study are:-

1. To compare different cytology staining methods on Boer goat spermatozoa morphology and morphometry.

2. To identify the best staining techniques for better semen evaluation.

The hypotheses of this study are different types of stain produces different dimension of goat sperms head and also the morphology and morphometry of the Boer goat spermatozoa.

REFERENCES

Aksoy, E., Aktan, T.M., Duman, S., Cuce, G. (2012).Assessment of spermatozoa morphology under light microscopy with different histologic stains and comparison of morphometric measurements. *Int. J. Morphol.,30(4)*:1544-1550.

Andraszek, K., Banaszewska, D., Czubaszek, M., Wójcik, E., Szostek, M.(2014). Comparison of different chromatin staining techniques forbull sperm. *Arch. Tierz.57*, 1–15

Banaszewska, D., Andraszek K., Czubaszek M., Biesiada-Drzazga, B. (2015). The effect of selected staining techniques on bull sperm morphometry. *Animal Reproduction Science, 159* , pp. 17-24.

Barth, A.D.&Oko, R.J. (1989). Abnormal Morphology of Bovine Spermatozoa. Iowa State

Björndahl, L., Söderlund, I., &Kvist, U. (2003). Evaluation of the one-step eosin-nigrosin staining technique for human sperm vitality assessment. *Human reproduction, 18(4),* 813-816. University Press, Ames. 285p.

Campbell, R. C., Hancock, J. L., Shaw, I. G. (1960). Cytological characteristics and fertilizing capacityof bull spermatozoa. *J Agrie Sci 55*: 91-99

Campbell,R.C, Dott, H. M. &Glover, T.D. (1956). Nigrosin eosin as a stain for differentiating live and dead spermatozoa. *J. agrie. Sci., Camb*. *48*, 1-8.

Coetzee, K., Kruger, T. F., Vandendael, A., de Villiers,A., Lombard, C. J. (1997). Comparison of two staining and evaluation methods used for computerized human sperm morphology evaluations. *Andrologia ;29*:133-135.

Chandler, J. E., Painter, C.L., Adkison, R.W., Memon, M.A. and Hoyt, P.G. (1988). Semen quality characteristics of dairy goats. *Journal of Dairy Science*, *71*:1638-1646.

Ciftci, H.B., Zülkadir, U., 2010. The correlation between bull sperm headdimensions and mitochondrial helix length. *J. Anim. Vet. Adv. 9,*1169–1172.

Coetzee, K., Kruger, T. F., Lombard, C. J. (1998).Predictive value of normal sperm morphology: a structured literature review. *Hum Reprod Update;4*:73-82.

Cummins, J.M. (1983). Sperm size, body mass and reproduction in mammals. In The Sperm Cell, pp. 395-398. Ed. J. André. MartinusNijhoff, The Hague.

Cummins, J. M., & Woodall, P. F. (1985). On mammalian sperm dimensions. *Journal of Reproduction and Fertility, 75(1),* 153-175.

De Vos, A., Van De Velde, H., Joris H, Verheyen, G., Devroey, P., Van Steirteghem, A. (2003). Influence of individual sperm morphology on fertilization, embryo morphology, and pregnancy outcome of intracytoplasmic sperm injection. *FertilSteril79*, 42-48.

Ferrari, S., Barnabe, V. H.,Zge, R. M.&Zogno, M. A. (2000). Effect of two ram sperm capacitating media on acrosome reaction and zona-free hamster oocyte penetration test. *Braz. J. Vet. Res. Anim. Sci., 37*: 147-158.

Gage, M. J.G. 1998. Mammalian sperm morphometry.*Proc. R. Soc. Lond. 265*:97-103.

García-Herreros, M., Aparicio, I. M., Barón, F. J.,García-Marín, L. J. & Gil, M. C. (2006). Standardization of sample preparation, staining and sampling methods for automated sperm head morphometry analysis of boar spermatozoa. *Int. J. Androl., 29(5):*553-63.

Gomendio, M., Roldan, E.R., 2008. Implications of diversity in sperm sizeand function for sperm competition and fertility. *Int. J. Dev. Biol. 52*,439–447.

Guzick, D. S., Overstreet, J. W., Factor-Litvak, P., Brazil, C.K., Nakajima, S. T., Coutifaris, C., Carson, S.A., Cisneros, P., Steinkampf, M.P., Hill, J. A., Xu, D., Vogel, D.L. (2001). Sperm Morphology, Motility, and Concentration in Fertile and Infertile Men. *N Engl J Med345*, 1388-1393

Hidalgo, M., Rodríguez, I., Dorado, J. (2006). Influence of staining and sampling procedures on goat sperm morphometry using the Sperm Class Analyzer. *Theriogenology66*, 996-1003

Immler, S., Saint-Jalme, M., Lesobre, L., Sorci, G., Roman, Y. &Birkhead, T. R. (2007). The evolution of sperm morphometry in pheasants. *Journal of Evolutionary Biology*.

Koonjaenak, S. (2006). Semen and sperm characteristics of Swamp buffalo (Bubalusbubalis) bulls for Artificial insemination in Thailand, in relation to season. Doctoral Thesis.Faculty of Veterinary Medicine and Animal Science, Swedish University of Agricultural Sciences.

ActaUniversitatisAgriculturaeSueciae*vol. 2006:114. Uppsala, Sweden. ISSN 1652-6880, ISBN 91-576-7263-6.* 53 pp.

Kuster, C. E., Singer, R. S. &Althouse, G. C. (2004). Determining sample size for the morphological assessment of sperm. *Theriogenology,61(4):*691-703.

Lingappa, H. A., Govindashetty, A. M., Krishnamurthy, A., Puttaveerachary, A. K., Manchaiah, S., Shimoga, I. C., Mallaradhya S.H., &Gowda, S. B. M. (2015). Quest for An Ideal, Simple and Cost-Effective Stain for Morphological Assessment of Sperms. *Journal of clinical and diagnostic research: JCDR, 9(10),* EC01.

Lu, C. D. (2001). Boer goat production: progress and perspective. In Proceedings of the 2001. International Conference on Boer Goats in China (pp. 9-17).

Malom, A.F., Gomendiom, M., Gardem, J., Lang-Lentonm, B., Solerm, A.J.,Roldanm, E.R.S., 2006. Sperm design and sperm function. *Biol. Lett. 2*,246–249.

Maree, L., du Plessis, S. S., Menkveld, R., van der Horst, G. (2010). Morphometric dimensions of the human sperm head depend on the staining method used. *Hum. Reprod. 25*:1369–1382.

Menkveld, R. (2007). The basic semen analysis. In: Male Infertility. Diagnosis and treatment, edited by S. Oehninger and T.F. Kruger, UK: Informa Healthcare, pp. 141–170.

Mota, P. C., Ramalho-Santos, J. (2006). Comparison between different markers for sperm quality in the cat: Diff-Quik as a simple optical technique to assess changes in the DNA of feline epididymal sperm. *Theriogenology;65*:1360–1375.

Nagvenkar, P., Zaveri, K., Hinduja, I. (2005). Comparison of the sperm aneuploidy rate in severe oligozoospermic and oligozoospermic men and its relation to intracytoplasmic sperm injection outcome. *FertilSteril84*, 925-931.

Nuti, D. L. C. (2013). Goat semen collection and processing..Retrieved from http://goatdocs.ansci.cornell.edu/Resources/GoatArticles/GoatGeneticsRepro/GoatSemenNuti.pdf.

Phillips N.J., McGowan M.R., Johnston S.D. & Mayer D.G. (2004).Relationship between thirty post-thaw spermatozoal characteristics and the field fertility of 11 high-use Australian dairy AI-sires.*Anim. Reprod. Sci. 81(1/2):*47-61.

Prathalingam,N. S., Holt, W.W., Revell, S.G.,Jones, S., Watson, P. F. (2006). The precision and accuracy of six different methods to determine sperm concentration. *J. Androl.27* 257- 262.

Rodriguez-Martinez, H. (2013). Semen evaluation techniques and their relationship with fertility. *AnimReprod*, *10*(3), 148-159.

Rodriguez-Martinez, H. (2006). State of the art in farm animal sperm evaluation. *Reproduction, Fertility and Development19*, 91-101.

Rodriguez-Martinez, H. (2003). Laboratory semen assessment and prediction of fertility: still utopia? *Reproduction in Domestic Animals38,* 312-318.

Saacke, R. G.(2008). Sperm morphology: Its relevance to compensable and uncompensable traits in semen. *Theriogenology70* 473-478.

Steven, A., Wilson, I. (1996). The Haematoxylin and Eosin. Theory and practice of histopathological techniques. 4th edition. Bancroft JD, Steven A. New York: Churchill Livingstone; *6:* 99-104.

Sundararaman, M.N. and Edwin, M.J. (2008). Changes in Motility Characteristics of Goat Spermatozoa during Glycerol-Equilibration and the Relevance to Cryopreservation. *Asian Journal of Cell Biology, 3:* 22-33.

Tartaglione, C.M., Ritta, M.N.(2004). Prognostic value of spermatological parameters as predictors of invitro fertility of frozen-thawed bull semen. *Theriogenology. 62*:1245-52.

Uven, M.., Can, B. & Saran, Y. (1998).Ultrastructural Changes in the Spermatozoa of Infertile Men. TurkiyeKlinikleri*J. Med. Res*., *16*:103-5.

Van der Horst, G., &Maree, L. (2010).SpermBlue®: A new universal stain for human and animal sperm which is also amenable to automated sperm morphology analysis. *Biotechnic&Histochemistry, 84(6),* 299-308.

Verstegen, J., Iguer-Ouada, M., Onelin, K. (2002). Computer assisted semen analyzers in andrologyresearch and veterinary practice. *Theriogenology, 57,* 149–179.

Watson, P. F. (1975). Use of Giemsa stain to detectchanges in acrosomes of frozen ram spermatozoa.*Vet Rec 97*: 12–15.