



UNIVERSITI PUTRA MALAYSIA

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

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FPV 2016 52

**COMPARISON OF DIFFERENT CYTOLOGIC STAINING TECHNIQUES
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MORPHOMETRY**

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A project paper submitted to the
Faculty of Veterinary Medicine, Universiti Putra Malaysia

In partial fulfilment of the requirement for the
DEGREE OF DOCTOR OF VETERINARY MEDICINE

Universiti Putra Malaysia,
Serdang, Selangor DarulEhsan.

MARCH 2016

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

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DEDICATIONS

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

My family,

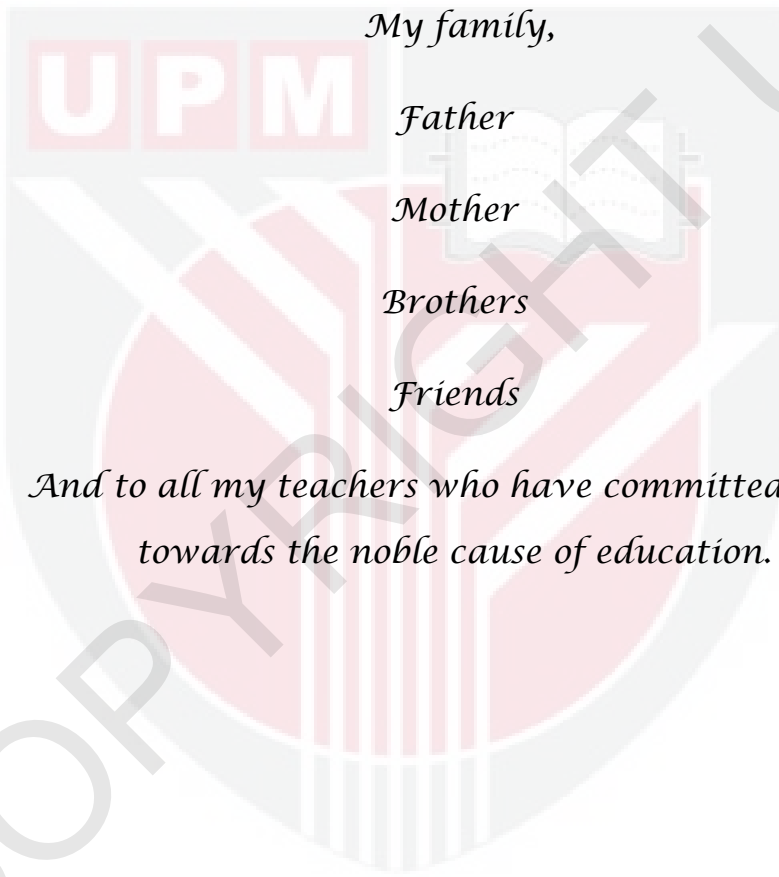
Father

Mother

Brothers

Friends

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



ACKNOWLEDGEMENTS

With a deepest appreciation and gratitude, I thank Allah S.W.T and all those who have made this project paper a reality.

To the persons that have assisted me throughout this project, I would firstly like to thank my project supervisor, Dr. Intan Shamehabinti Abdul Razak for her time, wisdom, expertise, support and guidance that she had granted me throughout the duration of this project, and my studies at the faculty.

Thank you to Dr. Hasliza, Dr. Mark, Prof. Dr. Abd Wahid Haron, and others for their help throughout the sample collection for this project. I would also like to thank A.P. Dr. Rosnina, Mr. Yap, Mr. Fahmi, Mr. Murthi, the staff of Histology, and Histopathology Lab for always lending me a helping hand when I needed it and not to forget, a special thanks dedicated to Prof. Dr. Mohd. Ariff for the guidance and knowledge.

Thank you to Ayu, Mira, Atty, Ida and all my classmates of DVM 2016, who assisted me directly or indirectly in this project.

Last but not least, my most heartfelt gratitude to my family for their love and support throughout my studies.

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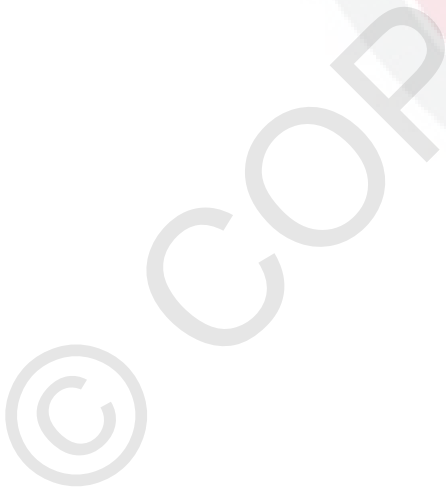
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LIST OF ABBREVIATIONS

AI	Artificial insemination
°C	Celcius
CASA	Computer-Assisted Sperm Analysis
cm	centimeter
DPX	dibutylphthalate xylene
DQ	Diff-Quik
EN	Eosin-Nigrosin
<i>et al.</i>	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



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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

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2016

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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

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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 morfologi sperma 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 is essential 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.

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