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BETWEEN HORNED AND POLLED, IS THERE A KARYOTYPIC DIFFERENCE IN SHEEP?

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BETWEEN HORNED AND POLLED, IS THERE A KARYOTYPIC DIFFERENCE IN SHEEP?

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CERTIFICATION

It is hereby certified that I have read this project paper entitled "Between horned and polled, is there a karyotypic difference in sheep?" by Nurul Liyana Binti Jamaluddin and in my opinion is satisfactory in terms of scope, quality, and presentation as partial fulfilment of the requirement for the course VPD 4999- Final Year Project.

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DEDICATIONS

This project paper is dedicated to my family:

Mother

Father

Siblings

and Friends

Thank you for all the support throughout this journey

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CONTENTS

LIST OF TABLES

LIST OF FIGURES

LIST OF ABBREVIATIONS

PHA	: Phytohaemagglutinin
PWM	: Pokeweed mitogen
ICNDA	: International Cytogenetic Nomenclature of Domestic Animal
2n	: Diploid number
NF	: Fundamental number
DVS	: Department of Veterinary Services
DNA	: Deoxyribonucleic acid
Rpm	: Revolutions per minute
KCL	: Potassium chloride

ABSTRAK

Abstrak daripada kertas projek yang dikemukakan kepada Fakulti Perubatan Veterinar untuk memenuhi sebahagian daripada keperluan kursus VPD 4999 - Projek Ilmiah Tahun Akhir

ANTARA BERTANDUK DAN TIDAK BERTANDUK, ADAKAH TERDAPAT PERBEZAAN KARIOTIP PADA BIRI-BIRI?

Oleh

Nurul Liyana Binti Jamaluddin

Mac 2017

Penyelia: Prof Dr. Rosnina Hj. Yusoff

Analisis kariotip telah dijalankan ke atas dua baka biri-biri, Merino (bertanduk) dan Dorper (tidak bertanduk) untuk membandingkan ciri-ciri kromosom mereka. Limfosit diekstrak darpada sampel darah dan dikulturkan dalam media RPMI 1640, yang ditambah dengan serum anak lembu dan penisilin-streptomisin. Kultur pendua telah dihasilkan, menggunakan mitogen, phitohemaglutinin (PHA) dan pokeweed (PWM) untuk merangsang pertumbuhan limfosit. Kultur yang yang telah menggunakan PWM menghasilkan penyebaran metafasa yang lebih berkualiti dan

lebih banyak berbanding kultur PHA. Kariotip telah dibina berasaskan prosedur oleh *International Cytogenetic Nomenclature of Domestic Animal* (ICNDA). Biri-biri domestik mempunyai nombor diploid (2n) 54 kromosom. Morfologi kromosom Merino dan Dorper tidak dapat dibezakan melalui kariotip konvensional. Kedua-dua jantan dan betina mempunyai kariotip yang terdiri daripada tiga pasang autosom submetasentrik dan 23 pasang autosome akrosentrik. Kromosom X dikenalpasti sebagai akrosentrik terbesar manakala kromosom Y dikenalpasti sebagai kromosom paling kecil. Nombor asas (NF) untuk kedua-dua baka biri-biri jantan dan betina ialah 60.

Kata kunci: Biri-biri, Merino, Dorper, bertanduk, tidak bertanduk

ABSTRACT

An abstract of the project paper presented to the Faculty of Veterinary Medicine in partial fulfilment of course VPD4999 – Project.

BETWEEN HORNED AND POLLED, IS THERE A KARYOTYPIC DIFFERENCE IN SHEEP?

By

Nurul Liyana Binti Jamaluddin

March 2017

Supervisor: Assoc. Prof Dr. Rosnina Hj. Yusoff

A karyotypic analysis was carried out on two breeds of sheep, Merino (horned) and Dorper (polled) to compare their chromosome features. Lymphocytes were extracted from blood samples and cultured in RPMI 1640 as a culture medium, supplemented with foetal bovine serum and penicillin-streptomycin. The cultures were made in duplicates, using phytohemagglutinin (PHA) and pokeweed (PWM) mitogens to stimulate lymphocytes growth. Cultures with PWM produce better quality and quantity of metaphase spreads compared with PHA. Karyotypes were constructed in accordance with the standard procedure of International Cytogenetic Nomenclature of Domestic Animal (ICNDA). The domestic sheep has a diploid number (2n) of 54. The chromosomes morphology of Merino and Dorper are indistinguishable based on their

conventional karyotypes established. Both males and females have karyotypes of three pairs of submetacentric and 23 pairs of acrocentric autosomes. The X chromosome is identified as the largest acrocentric while the Y chromosome is identified as the smallest chromosome. The fundamental number (NF) for the male sheep is 60 and the female is also 60.

Keywords: Sheep, karyotype, Merino, Dorper, horned, polled.

1.0 INTRODUCTION

1.1 Background

The domestic sheep, *Ovis aries* was first dometicated over 10,000 years ago for agricultural purposes (Haenlein, 2007). Undomesticated sheep typically have horns and horns are used by males to compete for mate during breeding season whereas in females the horns are used to compete for food during peri-parturient period (Coltman & Pemberton, 2004). The occurence of polled sheep is increasing as domestication continues; this is because the horned trait is not desirable for farmers and thus, selective breeding is practiced. In some sheep breeds, both sexes are either horned (e.g. Merino) or polled (e.g. Dorper) while in some breeds only the rams have horns (e.g. Rambouillet). Also, there are breeds that have both horned and polled strains. A comparison of the chromosome features of horned breeds like the Merino with those of Dorper (polled) which is overtly normal has not been carried out to date. Therefore, this study is carried out to compare the differences in the total number of chromosomes (diploid number, 2n), fundamental number (NF) and morphology of the chromosomes between horned Merinos and polled Dorpers.

1.2 Justification

To map out the karyotype to see the morphological differences in chromosomes between the horned and polled species of sheep.

1.3 Hypothesis

H_o: There are no differences between the karyotypes of Merino and Dorper.

H_A: There are differences between the karyotypes of Merino and Dorper.

2.0 LITERATURE REVIEW

2.1 Origin of the domestic sheep

Sheep was first domesticated about 10,000 years ago, at least in three separate times. They were believed to be originated from the wild mouflon (*Ovis gmelini*). Two wild population of mouflons still exist; they are the Asiatic mouflon and European mouflon (Hirst, 2016). Farmers have incorporated them into farms because of their ability to easily adapt to local environment. Sheep are reared mainly for meat, but they also provide milk, leather and wool. Throughout domestication, the morphological characteristics of sheep changed from horned to increase occurrence of polled trait, a decrease in body size and large population of younger stocks. These are the desirable traits selected by farmers through selective breeding.

2.2 Sheep breeds

There is a wide range of sheep breeds in the world with more than 1000 distinct sheep breeds. They are classified according to their purpose either for meat, milk or wool; the type of coat either wool or hair; the colour of their faces and/or by a specific physical or production characteristics. Sheep that are reared for meat purposes are usually those that produce medium or long wool. They account for about 15% of the world's sheep population. For the wool type they are further categorized as fine wool, medium wool, long wool and carpet wool. The popular breeds for fine wool are Rambouillet and Merino, for medium wool are Dorset and Suffolk, long wool are Romney and Border Leicester and the common carpet wool are Karakul and Navajo Churro. Hair sheep are mostly found in Africa and the Caribbean. They are covered with hairs and some have a mixture of hair and wool fibres that are shed naturally. The common breeds of hair sheep are Katahdin and Barbados Black Belly.

Dorper is a breed of sheep that originated from South Africa. They are produced as a result of crossing Dorset Horn with Blackheaded Persian sheep back in the 1930s. Dorset Horn, as the name implies, is horned while the Blackheaded Persian sheep is polled for both sexes. Through selective breeding most of Dorper individuals are polled because of its dominance over horned. There are two distinct colorations of Dorper, one with a black head and another with white head. Dorper is a favourite among farmers because of its good fertility, good body length and a short covering of hair and wool. Besides that, they also have good mothering abilities, are highly hardy and adaptable. They are bred primarily for meat and can produce good quality carcasses. On top of that, are highly demanded for their thick and highly valuable sheepskin.

Merino is a breed which originated from Spain; known as far back in the 12th century. They are prized for their fine wool quality. Merino are imported into Australia and through selective breeding has produced Merinos that have superior fine quality of wool.

2.3 Sheep industry in Malaysia

The livestock industry plays a major role in Malaysian economy with poultry, eggs and pork as the main contributors while small ruminant (sheep and goats) play a minor role in the industry. Information from Department of Veterinary Service (DVS) in 1999 revealed that mutton production only contributes about 0.2% of the total gross output value of livestock. However, throughout the years, the trend of mutton consumption is increasing, with it being an acceptable choice of meat to all races in Malaysia and about 72% of the population of Peninsular Malaysia consume mutton

(Mohamed, Chiew, & Chong, 1995). One of the reasons of the sheep industry being left far behind is that majority of the farms are still owned by individual farmers and also the total percentage of small ruminant ranchers in Malaysia is only about 16% (MOA, 2011). Some of the problems faced by small ruminant farmers are feed price, breed and stock, price of meat and live small ruminants, ranchers' skill and management and capital for rearing small ruminants (Yusoff et al., 2016). Sheep rearing usually is carried out in villages, in rubber estates and oil palm plantations, fruit orchards, rice fields and vacant land. Majority of the sheep population are reared for meat consumption. The sheep market supplies the Muslim community for their ritual slaughter requirement for example during the celebration of Haj and aqiqah along with the Indian community but for a different ritual.

2.4 Inheritance of horn trait in sheep

The presence of horns is controlled by a single autosomal locus, Horns (Ho) with three alleles which are Ho^P, Ho^+ and Ho^L. The allele Ho^P is responsible for the occurence of polledness, which is incompletely dominant to the other two allele in rams and almost completely dominant in ewes. Ho^+ produce horns in both sexes and Ho^{h1} produces sex-limited horns (Montgomery et al., 1993). Based on the combination of alleles inherited and sex, this will determine the phenotype expressed by the sheep (Pickering et al., 2009). In rams, a further autosomal locus known as scurs affect the growth of horns. The scur allele interact with the Ho locus resulting in the growth of scurs or abberant horns. The short knobs or scurs may or may not be attached to the frontal bone (Montgomery et al., 1993). There is a rare occurrence where some sheep express a four-horned trait and they are mainly found in short-tailed

prehistoric sheep in Northwest-Europe. This trait is dominant over ordinary two-hornedness but polledness is dominant over two- as well as four-horned sheep (Dyrmundsson, 2005).

2.5 Sheep chromosome and karyotype

The term chromosome was first used by Waldeyer (1888), taken from Greek words for colour (chroma) and body (soma) because of their affinity to colourful dyes. Chromosomes are the most important component in a cell, located inside the nucleus of an animal or a plant cell. These thread-like structures are made up of proteins called histones and molecules of deoxyribonucleic acid (DNA). The passing of DNA evenly between cells during cell division is very important and the chromosomes play a major role during this process. Mistakes could happen during cell division resulting in the incorrect number of chromosomes or change in chromosome morphology because of deletion, duplication, insertion or translocation. Some of the results of chromosome alterations can be seen phenotypically, altering the morphology of a certain body parts or structures.

Chromosomes occur in pairs and compose of autosomes and one pair of sex chromosomes that is XX in females and XY in males. The shape of chromosomes changes throughout the process of cell division and are best seen at metaphase where they are thick and filamentous. There is centromere along the length of a chromosome; dividing it into two parts, each is an arm. The shape of chromosomes is determine by position of the centromere. Telocentric chromosome is when the centromere is located at the proximal end, acrocentric is when the centromere is located close to the end, producing a very short (p) arm and another long arm (q). Sub-metacentric is when the

centromere is located close to the centre producing unequal arms and lastly metacentric is when the centromere is located at the centre of chromosome producing equal arms.

Karyotype is defined as the chromosomes arrangement taken from a cell during metaphase, based on a universally agreed layout scheme that is species specific. They are arrange based on shape, from metacentric/sub-metacentric to acrocentric/telocentric and decreasing in size (Chowdhary & Raudsepp, 2005). There are several ways to carry out karyotyping and the most commonly done is conventional karyotype. A more detailed chromosome analysis can be done using banding techniques such as G-banding, Q-banding, C-banding or R-banding.

3.0 MATERIALS AND METHOD

3.1 Animals

In this study, four animals were used which are a pair of Merino sheep (one male and one female) and a pair of Dorper sheep (one male and one female). These animals belong to a farm located about 23km from Universiti Putra Malaysia (UPM).

3.2 Chromosome preparation

3.2.1 Blood culture

Blood samples from one male and one female from both breeds (Merino and Dorper) were collected from the jugular vein and collected in heparin tubes. Prior to blood collection, the wool at the jugular area was clipped and swabbed with 80% alcohol. The heparin tubes stopper was swabbed with alcohol prior to and after blood collection. After blood collection, the samples were agitated by hand and put in test tube rack in an ice box, surrounded with ice pack inside. The samples were immediately transported back to UPM for culture.

In the laboratory, the blood samples were centrifuged at 1800 rpm for 10 minutes in a clinical centrifuge (Kubota 2100, Japan) to obtain the buffy coat containing leucocytes. The buffy coat was aspirated using a sterile pipette and five drops were put into a culture flask containing 8ml RPMI 1640 culture medium supplemented with 2ml of 15% feotal bovine serum, 0.1ml antibiotic (Pen-Strep) and 0.1ml 1% mitogen. The cultures were prepared in duplicates as two types of mitogen were used, phytohemagglutinin (PHA Gibco) and pokeweed (PWM Gibco). The cultures were agitated gently and put into incubator at 38°C for 72 hours. Twice a day, the cultures were agitated gently, once in the morning and another in the evening.

3.2.2 Harvesting the culture

Fifty minutes before harvest, 0.1ml of colcemid solution is added into each flask and was gently shaken. At the end of the 72 hours incubation period, the blood cultures were transferred into 15ml centrifuge tubes and spun at 1800 rpm for 8 minutes. The supernatant was discarded leaving 2ml supernatant and button. Then, 6ml of prewarmed potassium chloride solution (0.075 M KCL) was added into each tube and the tubes were incubated for another 20 minutes. After incubation, the tubes were centrifuged again for 8 minutes. All the supernatant will be discarded leaving behind the button. The cell button was broken using a pasteur pipette and 6ml Carnoy's fixative was added. The tubes were spun again and the supernatant was discarded. The fixation procedure was repeated 3 times. At the end of the 3rd spin, 3ml of fresh fixative was added into each tube and the cell button and fixative were gently mixed using pasteur pipette.

3.3 Slide preparation

Before using the glass slides (with frosted ends), the glass slides were immersed and cleaned in 70% alcohol overnight, then immersed in double distilled water and chilled in refrigerator (4°C). Two drops of the cell suspension were dropped onto the prechilled glass slide,the first drop was allowed to dry first before dropping the second suspension. Then the slides were held over steam emanating from boiling water for 30 seconds and stained with 10% Giemsa for 5 minutes.

3.4 Construction of karyotype

Slides were examined under a Zeiss flourescence microscope and cell with a good metaphase spread was photographed using Technical Pan film. The number of chromosomes were counted from 100 cell spreads to determine the diploid number (2n) followed by determination of the fundamental number (NF) through counting the total number of short (p) and long (q) arms in the karyotype. For construction of the karyotype, the chromosomes were cut from prints and arranged according to the International System for Cytogenetic Nomenclature of Domestic Animals (ISCNDA).

4.0 RESULTS

4.1 Chromosome constitution

The distribution of chromosome number per cell for Merino and Dorper are shown in Table 1.0 and Table 2.0 respectively. The distribution was counted based on a combination series of lymphocytes cultures in phytohemagglutinin (PHA) and pokeweed (PWM) mitogen.

Merino	Number of chromosome				
	52	**53**	**54**	**55**	Total
Male	19	20	57	4	100
Female	31	16	48	5	100

Table 1

Dorper	Number of chromosome				
	52	**53**	**54**	**55**	Total
Male	27	17	53	3	100
Female	32	16	45	7	100

Table 2

The distribution of chromosomes per cell for Merino male and female are highest at 54 which are 57% and 48% respectively. As for Dorper male and female, the highest chromosome number is at 54 which are 53% and 45% respectively. The second highest number of chromosomes is 52, indicating loss of a pair of chromosomes. The chromosomes number count of 53 and 55 could be due to artefacts

during preparation or missing of a chromosome or addition of a chromosome from the neighbouring cell spreads.

4.2 Chromosome morphological difference

The morphological difference between the two breeds of sheep (Merino and Dorper) were indistinguishable based on the conventional karyotype established. Both males and females have karyotypes of three pairs of submetacentric and 23 pairs of acrocentric autosomes. The X chromosome is identified as the largest acrocentric while the Y chromosome is identified as the smallest chromosome. The fundamental number of Merino and Dorper males is 60 and likewise for the females. The fundamental number is counted based on the total number of arms present in the karyotype.

The morphological characteristics of chromosomes of both sexes are shown in Table 3.0.

	AUTOSOMES		SEX CHROMOSOME		TOTAL NUMBER
	SM	AC	X	Y	
Male	6	46	AC	AC	54
Female	6	46	AC	-	54

Table 3

SM: Submetacentric

AC: Acrocentric

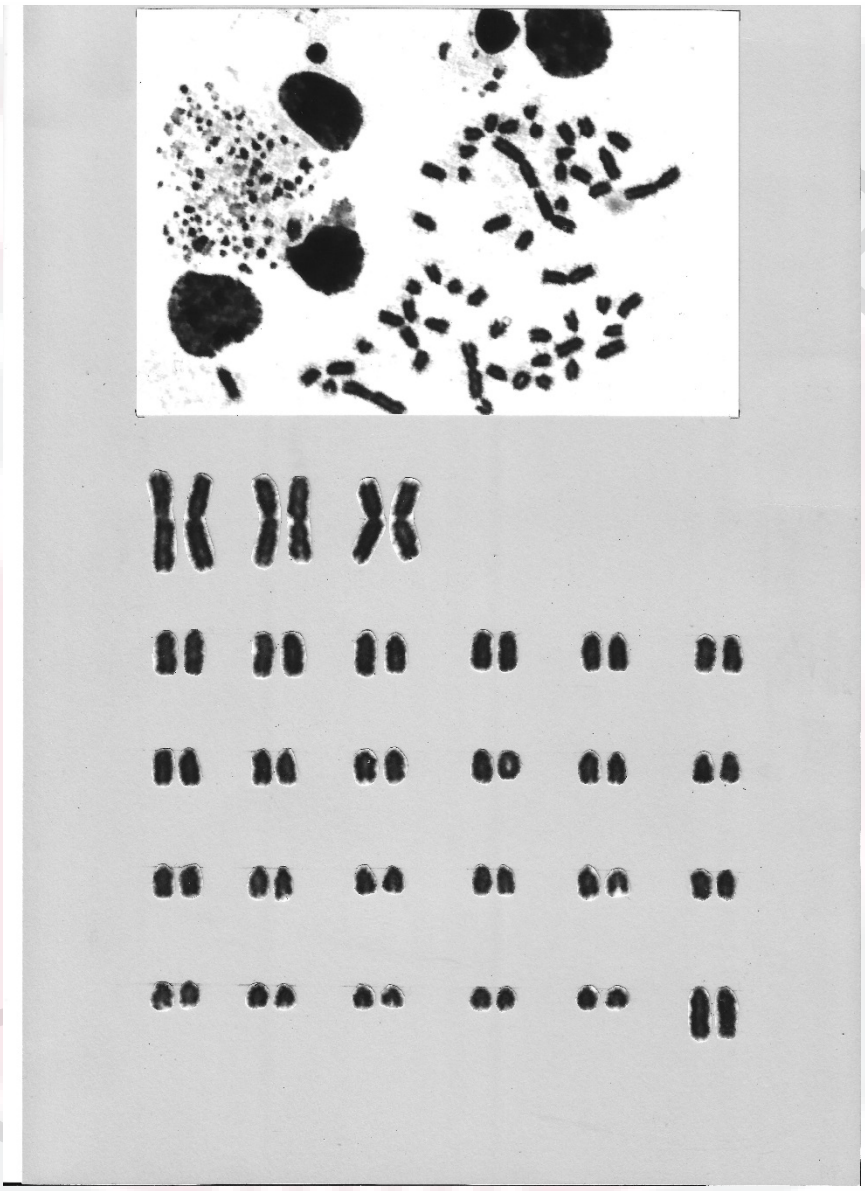

Figure 1: Karyotype of Dorper female

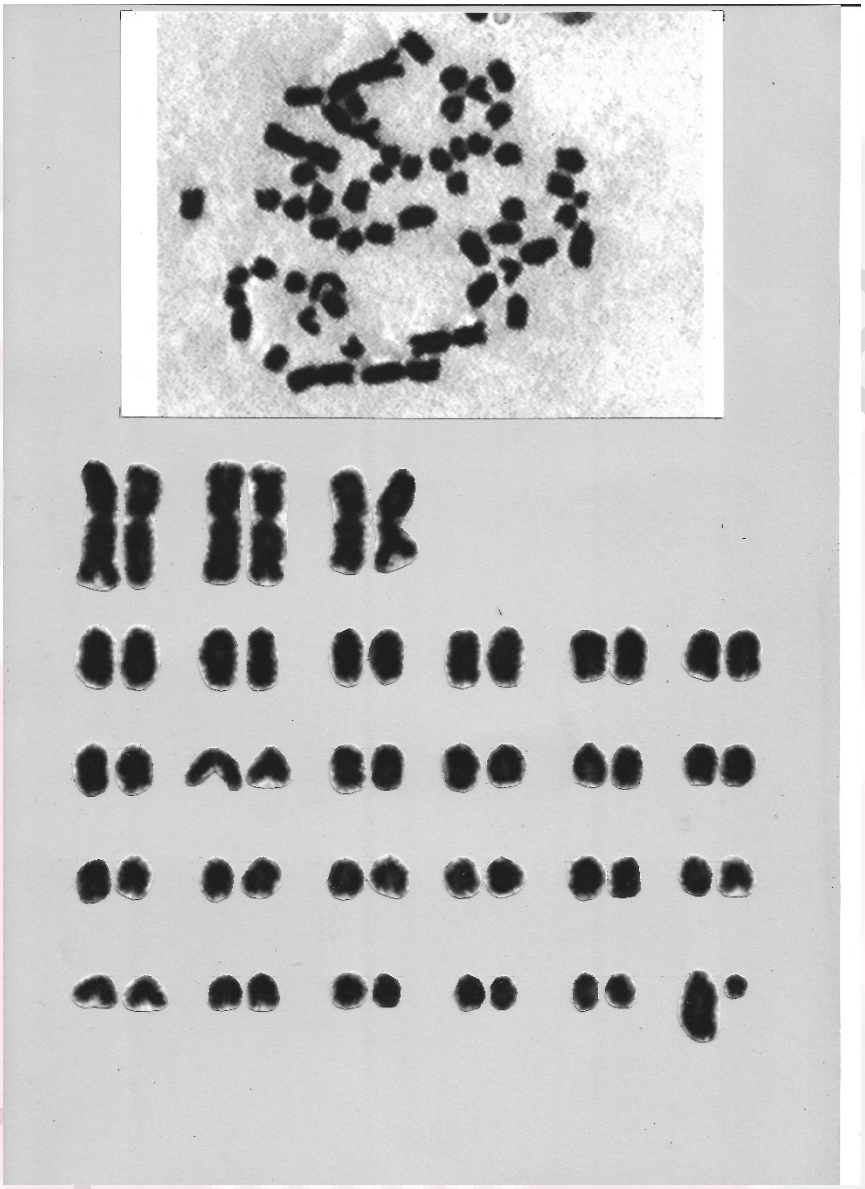

Figure 2: Karyotype of Dorper male

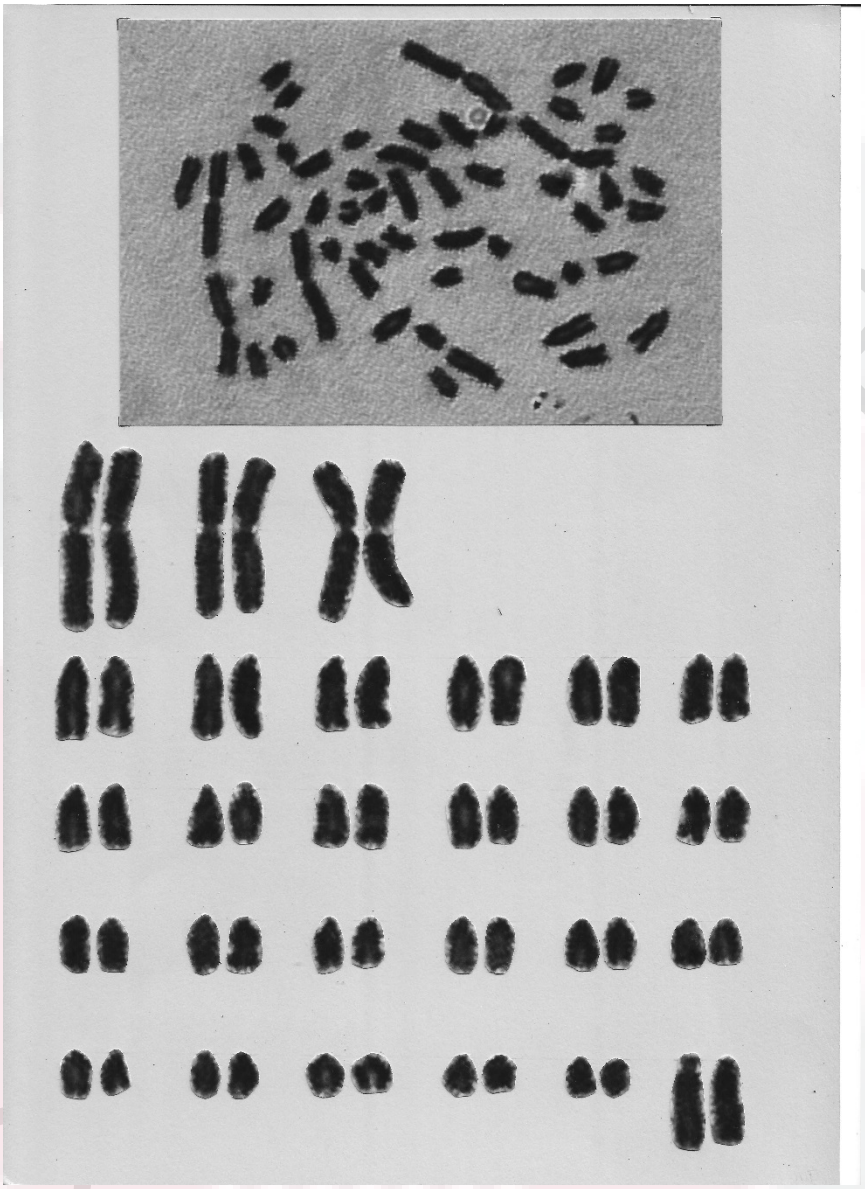

Figure 3: Karyotype of Merino female

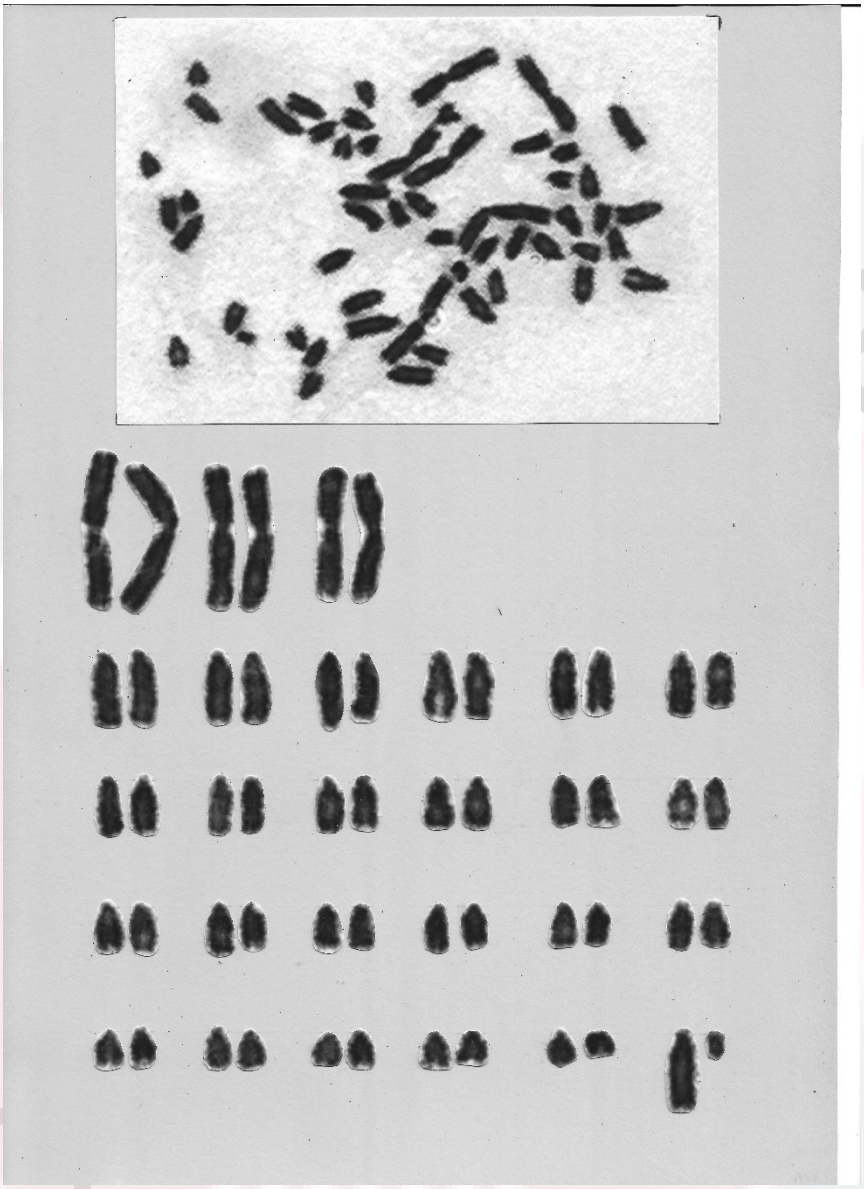

Figure 4: Karyotype of Merino male

4.3 Mitogens

Cultures for each animal were made in duplicates using two different mitogens, phytohaemagglutinin (PHA) and pokeweed (PWM). Cultures with PWM produced good quantity and quality of metaphase spreads whereas cultures with PHA resulted

in metaphase spreads with a lot of clumping and overlapping. Figure 1.0 shows an example of a good metaphase spread from PWM and Figure 2.0 shows a poor spread from PHA.

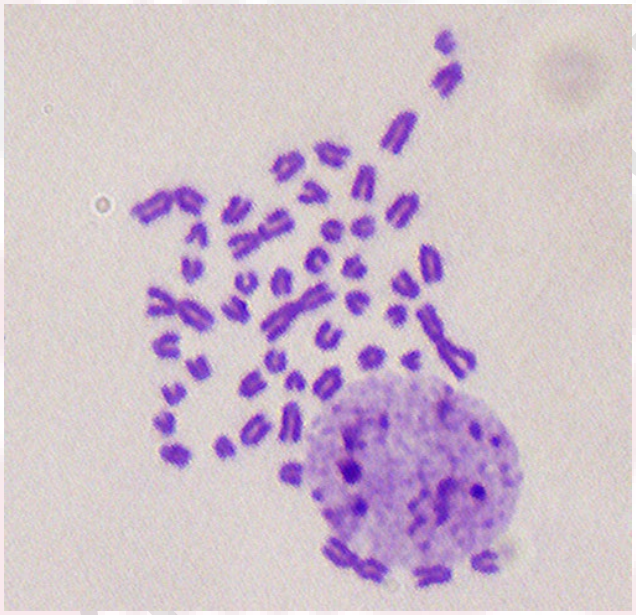

Figure 5: Well spread chromosomes

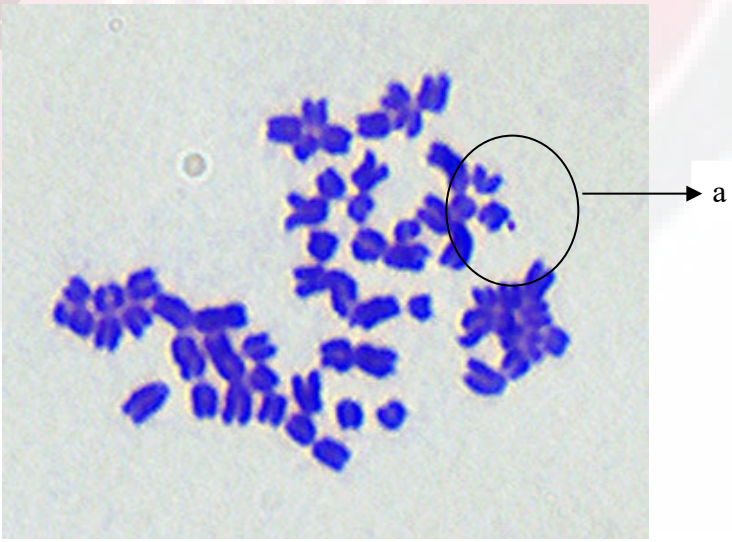

Figure 6: Chromosomes are close to each other with some overlapping (a)

5.0 DISCUSSION

5.1 Conventional karyotype Merino and Dorper

The chromosome number for domestic sheep (*Ovis aries*) is 2n=54. In some occasions, chromosomal translocation can occur to produce chromosome number of less than 54. This diploid number of chromosomes evolved either directly form a 2n = 54 stock or from a polyphyletic origin of 2n = 54, 56, and 58 genotypes (Bunch & Foote, 1977). Sheep in general have a variable set of chromosomes that is different in morphology (Ahmad & Khan, 2007). Wild sheep have different sets of chromosome number, the snow sheep (*Ovis nivicola*) has 2n = 52, the mouflon sheep (*Ovis orientalis*) and the North American wild sheep (*Ovis canadensis*) have 2n = 54, the Arkhar/Argali sheep (*Ovis dalli*) has 2n = 56 and the Urial sheep (*Ovis vignei*) has 2n = 58 (Bunch, 1978). The domestic sheep was postulated to be a descendant of the Asiatic Mouflon (*Ovis orientalis*) of the Western and Southwest Iran; this is later confirmed by mitochondrial DNA sequence variations (Hiendleder *et al.*, 1998).

Based on the result of this study, the chromosome number (2n) of Merino and Dorper is 54 as established by previous studies. The karyotype consists of three pairs of sub-metacentric and 23 pairs of acrocentric autosomes. The X chromosome is the largest acrocentric and the Y chromosome is the smallest chromosome as reported by Hansen (1973). Based on the karyotype established, the Y chromosome is too small to identify its morphology and thus is thought to be an acrocentric. The fundamental number of both male and female is the same which is 60.

5.2 Effect of phytohaemagglutinin (PHA) and pokeweed (PWM) mitogens

Mitogen is a substance that induces mitosis. In this study, two types of mitogens were used; phytohaemagglutinin (PHA) and pokeweed (PWM). Based on the results of this study, cultures with PWM produced a better quality and quantity metaphase spreads. Cultures with PHA mostly produced poor quality spreads. PHA is a lectin derived from red kidney bean (*Phaseolus vulgaris*) and has an affinity towards T-cell activation and contain potent, cell agglutinating and mitogenic activities (Movafagh, Heydary, Mortazavi-Tabatabaei, & Azargashb, 2011). PWM is an abstract from *Phytolacca americana* that have affinity to both T-cell an B-cell activation. The molecular weight of PWM is much lower than PHA (Brittinger, Hirschhorn, Hirschhorn, & Weissmann, 1969). According to a study by Schwarz (1968), by comparing rat lymphocyte cultures with and without PWM, it revealed that cultures with PWM showed a higher number of transformed or enlarged cells after 72 hours. Also, when compared to PHA cultures, it was observed that a shorter period of time was needed for transformed cell to appear and a greater number of mitotic figures was seen in PWM cultures.

5.3 Recommendations

In my recommendation for future studies, banding techniques can be done, giving rise to alternating light and dark regions called bands. Banding techniques can uniquely identify individual chromosomes, even though the gross morphology is the same. Some of the examples of banding techniques are G which is Giemsa banding, R-banding which is a reverse G-banding and C-banding in which the centromere is

stained. The most commonly done banding technique is G-banding in which the slides are treated with trypsin, the G-bands will appear in the sulphur rich protein areas.

6.0 CONCLUSION

In conclusion, the diploid number of chromosome (2n) and fundamental number (NF) of both breeds are the same which are 2n = 54 and NF = 60. Chromosomes morphology of Merino (horned) and Dorper (polled) are indistinguishable based on conventional karyotypes established.

7.0 APPENDICES

A. Hypotonic solution (0.075M KCL)

1. Add 5.59 g Potassium chloride in 1.0 L distilled water.

B. Giemsa solution (10%)

1. Measure 10 mL of Giemsa and mix with 90 mL PBS solution. Filter with #9 filter paper.

C. Carnoy's fixative

1. Mix 100 mL of acetic acid and 300 mL of methanol in a measuring cylinder.

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