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JUDUL: A STUDY ON GENERAL SEMEN CHARACTERISTICS OF DORPER RAM AND THE EFFECT OF ROOM AND CHILLED TEMPERATURE ON SPERM PROGRESSIVE MOTILITY

IJAZAH: DEGREE OF BACHELOR OF AGRICULTURE SCIENCE WITH HONOURS

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A STUDY ON GENERAL SEMEN CHARACTERISTICS OF DORPER RAM AND THE EFFECT OF ROOM AND CHILLED TEMPERATURE ON SPERM PROGRESSIVE MOTILITY

SITI NUR NADIA BT. ABDUL RAZAK

DISSERTATION SUBMITTED IN PARTIAL FULFILLMENT OF THE REQUIREMENTS FOR THE DEGREE OF BACHELOR OF AGRICULTURE SCIENCE WITH HONOURS

LIVESTOCK PRODUCTION
SCHOOL OF SUSTAINABLE AGRICULTURE
UNIVERSITI MALAYSIA SABAH
2010

DECLARATION

I hereby declare that this dissertation is based on my original work except for citations and quotations which have been duly acknowledged. I also declare that no part of this dissertation has been previously or concurrently submitted for a degree at this or any other university.

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ABSTRACT

A study was conducted at Livestock Strategic Research Centre, Malaysian Agricultural Research and Development Institute (MARDI), Kluang, Johore. The aims of this study were to observe the general semen characteristics of Dorper ram and the effect of storage at room and chilled temperature on sperm progressive motility. For this purpose, six Dorper rams were selected. All the rams were individually subjected to one week of study and were scheduled for semen collection twice weekly using a teaser ewe. In each session, the reaction time was recorded. Semen was collected using an artificial vagina (AV) and was evaluated for volume, gross wave motility, sperm progressive motility, sperm concentration, live sperm and abnormal sperm. Each semen sample was diluted with extender divided into two tubes. One tube was stored at 5°C and the other at 26°C for seven days. The mean of reaction time, semen volume, wave pattern, sperm progressive motility percentage, sperm concentration, live sperm and abnormal sperm were 13.3±1.9 sec, 1.03±0.1 ml, 4.0±0.4, 69.2±7.8%, 2.44±0.4 $x10^9$ sperm/ml, 76.0±2.4% and 14.4±7.5%, respectively. On the third day of storage, it was observed that the semen that were stored at 26°C lost its progressive motility to 0% while semen stored at 5°C were observed with 50% progressive motility. The semen stored at 5°C lost its progressive motility to 0% on the sixth day of storage. It was concluded that the general semen characteristics of Dorper ram was within normal range and there was no significant difference on reaction time and semen quality between first and second collection. For the diluted semen, there was significant difference on sperm progressive motility percentage at 5°C in which the length of period for the sperm to lose its progressive motility percentage to 0% was longer compared to semen sample stored at 26°C.

Key words: Dorper ram, semen characteristics, temperature, sperm progressive motility

KAJIAN TENTANG CIRI-CIRI AM AIR MANI BIRI-BIRI PEJANTAN DORPER SERTA KESAN SUHU BILIK DAN SUHU PETI PENYEJUK TERHADAP PERGERAKAN PROGRESIF SPERMA

ABSTRAK

Kajian ini dijalankan di Pusat Penyelidikan Ternakan Strategik, Pusat Penyelidikan Pertanian dan Institut Pembangunan (MARDI), Kluang, Johor. Tujuan kajian ini adalah untuk membuat pemerhatian terhadap ciri-ciri am air mani biri-biri pejantan Dorper serta kesan suhu bilik dan suhu peti penyejuk terhadap pergerakkan progresif sperma. Bagi tujuan ini, enam pejantan Dorper telah dipilih. Kesemua enam pejantan adalah terlibat secara individu sepanjang tempoh seminggu kajian dan proses pengumpulan air mani dilakukan dua kali seminggu dengan menggunakan betina pembiang. Dalam setiap sesi, masa naik biang pejantan dicatat. Air mani dikumpul dengan menggunakan vagina tiruan (AV). Air mani dikaji dalam menentukan jumlah air mani, corak gelombang pergerakkan, pergerakkan progresif sperma, kepekatan sperma, sperma yang hidup serta sperma yang tidak normal. Kesemua air mani yang dikumpul dicairkan dengan cecair pelanjut. Setiap sampel air mani diasingkan ke dalam dua tabung uji. Satu tabung uji disimpan pada suhu 26°C dan satu disimpan pada suhu 5°C selama tujuh hari. Nilai purata bagi masa naik biang, jumlah air mani, corak gelombang pergerakkan, pergerakkan progresif sperma, kepekatan sperma, sperma yang hidup serta sperma yang tidak normal adalah 13.3±1.9 saat, 1.03±0.1 ml, 4.0±0.4, 69.2±7.8%, 2.4±0.4 $x10^9$ sperma/ml, 76.0±2.4% dan 14.4±7.5%, secara tertib. Pada hari ketiga penyimpanan, pemerhatian menunjukkan peratus pergerakkan progresif sperma bagi sampel air mani yang disimpan pada suhu 26°C telah berkurang sehingga 0%, manakala peratus pergerakkan progresif sperma bagi sampel air mani yang disimpan pada suhu 5°C adalah 50%. Peratus pergerakan progresif sperma bagi sampel air mani yang disimpan pada suhu 5°C berkurang sehingga 0% pada hari keenam penyimpanan. Kesimpulan bagi kajian ini adalah ciri-ciri am air mani biri-biri pejantan Dorper adalah normal dan tiada perbezaan bagi masa naik biang serta ciri-ciri am air mani pejantan Dorper antara sesi pengumpulan pertama dan kedua. Manakala, bagi sampel air mani ynag dicairkan, pemerhatian menunjukkan suhu 5°C memberi kesan terhadap peratus pergerakan progresif sperma di mana jangkamasa penurunan peratus pergerakan progresif sperma adalah lebih panjang berbanding apabila disimpan pada suhu 26°C.

Kata kekunci: Pejantan Dorper, ciri-ciri air mani, suhu, pergerakkan progresif sperma

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AI	Artificial Insemination
AV	Artificial Vagina
Co	Cobalt
Cu	Copper
g	Gram
GPC	Glycerylphosphorylcholine
I	Iodine
MARDI	Malaysian Agriculture Research and Development Institute
Mn	Manganese
MOS	Month of sheep
mt.	Metric tonne
sec	Seconds
Sperm/ml	Sperm per millilitre
SPSS	Statistical Package for Social Science
Zn	Zinc

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3.1	Sperm concentration	26

$$\text{Sperm concentration } (\times 10^9 \text{ sperm/ml}) = \frac{(A + B)}{2} \times 5 \text{ squares} \times 10^4 \times 10^3 \times V$$

A = Number of sperm on first chamber of haemacytometer
B = Number of sperm on second chamber of haemacytometer
5 squares = 5 squares that involved in the sperm count
10^4 = Factor of 10000 sperm
10^3 = Semen dilution factor 1: 1000
V= Volume of the semen sample

CHAPTER 1

INTRODUCTION

1.1 Overview of Sheep Industry in Malaysia

Sheep industry was introduced in Malaysia since the past decades. However, it could not become a popular industry in Malaysia as it was formed on a small scale and managed traditionally. There is inadequate number of quality sheep breeds problem exist here in Malaysia which contributed to slow development of the sheep industry. According to Mohamed *et al.* (2009), the sheep industry in Malaysia was at critical level in which sheep population consisted of only 115,500 heads in the year 2004 with 8.3% self sufficiency level (Appendix A). In the mean time, the market demand for mutton has increased over the years and is expected to continue due to the increase in Malaysian population and the change in lifestyle. As a result of that, importation of mutton had been done through these years to meet the surplus demands due to our low production.

1.2 Dorper Sheep

Dorper sheep was first introduced in Malaysia in the year 2007 at Malaysian Agriculture Research and Development Institute (MARDI) station Sg. Baging (Mohamed *et al.*, 2009). In the early year of 2009, 220 heads of Dorper sheep were brought into Malaysia for the evaluation of adaptability and performance under Malaysian climate (Suriya *et al.*, 2009). Dorper is an indigenous South Africa breed and was developed through crossing of Blackhead Persian with Dorset Horn (Cloete *et al.*, 2000). It has high average daily gain, well adapted to variety of climate, good carcass quality and had a very even temperament (Mohamed *el al.*, 2009; Julie Marzlinda *et al.*, 2009).

1.3 Importance of Ram in Breeding Programme

It is important to have rams breed stock that able to successfully mate in any breeding programme in order to transmit the desirable characteristic traits. Greater selection intensity is required in selection of herd ram to achieve the desired level of genetic improvement. Reproduction is considered as primary trait of economical importance in animal production systems. Moreover, it was clear that ram fertility contributed to reproductive efficiency and herd production (Leite-Browning, 2009).The pregnancy rate of ewe using Artificial Insemination (AI) was 66.66% which means that AI might be a solution for farmers who want to produce crossbred sheep without need to buy a ram. AI may also lead to a faster improvement of the local breed. Fertility examination is needed to identify potential rams so that ewes can have the best chance of conceiving to service (Seaman, 2004).

1.4 Justification

Dorper sheep was thought as one potential breed that might be able to boost the sheep industries and overcome the inadequate quality and fertile breed problem in Malaysia. Ram played an important role in the genetic improvement of sheep breeding through natural mating or AI. This study emphasized on evaluation of the Dorper ram fertility status, thus, selection can be made in order to perform an effective breeding program.

1.5 Objectives

The aims of this study were:

1. To evaluate the general semen characteristic of Dorper ram.
2. To determine the effect of room and chilled temperature on sperm progressive motility.

CHAPTER 2

LITERATURE REVIEW

2.1 Dorper ram

Dorper breed was a composite breed, combined the hardiness of Black-headed Persian with mutton production capability of the Dorset Horn. Dorper was an adaptable sheep breed, capable of maintaining acceptable levels of production under a wide variety of conditions. The previous study stated that epididymal sperm concentration of Dorper lambs rose markedly after an age of 140 days and mean sperm counts ($x10^9$) were at 0.1 at an age of 112 days, 0.2 at 140 days, 16.6 at 168 days, 27.9 at 196 days and 40.6 at 365 days (Cloete *et al.*, 2000). Therefore, it was clear that Dorper lamb and ram were able to fertilize ewes from quite an early age. Cloete *et al.* (2000) also noted that the Dorper testicular weight and the diameter of the seminifirous tubules increased with age for instance at the age of 365 days, testis weight averaged 303.8 g, epididymal weight averaged 54.1 g and the diameter of the semenifirous tubules averaged 209.4 µm. Moreover, Dorper rams were heavier and grew faster than Dorper ewes.

2.2 Ram Reproductive System

The male reproductive system consists of scrotum, spermatid cords, testes, accessory glands, penis, prepuce and the male duct system. The major functions of each organ in male reproductive system were presented in table 2.1. There are four accessory glands involved in providing liquid vehicle for the transport of sperm which are seminal vesicles, prostate gland, bulbourethral glands and urethral glands (Hafez, 2000).

Table 2.1 Reproductive organs of the male with their major functions

Organs	Function(s)
Testis	Production of spermatozoa
	Production of androgens
Scrotum	Support of the testes
	Temperature control of the testes
	Protection of the testes
Spermatic cord	*Support of the testes*
	Temperature control of the testes
Epididymis	Concentration of spermatozoa
	Storage of spermatozoa
	Maturation of spermatozoa
	Transport of spermatozoa
Vas deferens	Transport of spermatozoa
Urethra	Transport of semen
Vesicular glands	Contributes fluid and inorganic ions to semen
Prostate gland	Contributes fluid and inorganic ions to semen
Bulbourethral glands	Flushes urine residue from urethra
Penis	Male organ of copulation
Prepuce	Encloses free end of penis

Source: (Bearden and Fuquay, 1980)

Testosterone is a steroid hormone stimulating development that is needed for the development of sex characteristics, normal mating behaviour, function of the accessory glands, production of spermatozoa, maintenance of the male duct system and optimum conditions of spermatogenesis, transport of spermatozoa and deposition of spermatozoa into female tract (Bearden and Fuquay, 1980). Table 2.2 showed the development of reproductive tract accordingly to the age of ram. At the age of four month old, sperm already exist in ejaculate of a ram. Therefore, it was not recommended to be used for mating. Ideal age of ram that known as sexually matured was at the age of six month old.

Table 2.2 Development of the reproductive tract in ram (weeks)

Development	Weeks
Primary spermatocytes in seminiferous tubules	12
Sperm in seminiferous tubules	16
Sperm in caudal epididymis	16
Sperm in ejaculate	18
Completion of separation between penis and penile part of prepuce	>10
Age at which animal can be considered sexually mature	>24

Source: (Hafez, 2000)

2.3 Semen and Its Components

Semen consists of spermatozoa and seminal plasma (Bearden and Fuquay, 1980). Spermatozoa were supplied by epididymis and vas deferens while seminal plasma was supplied by accessory glands; vesicular glands, prostate gland and bulbourethral glands. According to Faezah (2005), semen was composed of approximately 10% of spermatozoa and 90% of seminal plasma.

2.3.1 Spermatozoa

Bearden and Fuquay (1980) stated that, theoretically 50% of the spermatozoa in a given ejaculate contained X chromosomes and 50% contained Y chromosomes, which on a population basis would result in equal numbers of male and female offspring. Approximately 80 to 90% of the spermatozoa in semen are expected to be progressively motile with an average speed of 6mm per minute.

The normal spermatozoa composed of a head, and a tail which divided into a mid-piece, main-piece and end piece. Spermatozoa must develop and retain five characteristics; metabolism, progressive motility, enzymes, proper distribution of lipids in the plasma and acrosomal membranes, and proteins in the plasma membrane critical for the survival of the spermatozoa in the female reproductive tract. All the characteristics are important for spermatozoa to be successfully fertilized an oocyte.

2.3.2 Seminal Plasma

Seminal plasma was stated as fluid portion of semen (Zaidi, 2006) that served as buffered, nutrient medium for the spermatozoa, and served as carrier and protector to the spermatozoa in natural mating (Bearden Fuquay, 1980). Seminal plasma contains sufficient environmental agents to maintain ejaculated sperm for relatively short periods of time (Gomes, 1977). Table 2.3 showed chemical composition in seminal plasma that act as nutrient medium in maintaining spermatozoa fertility.

Table 2.3 Average chemical composition of semen in ram

Constituents (Mg/cc)	A	B
Fructose	250	150-600
Sorbitol	72	26-120
Glycerylphosphorylcholine (GPC)	1650	1600-2000
Inositol	12	10-15
Citric acid	140	137
Sodium	190	78
Potassium	90	23
Calcium	11	1.9
Magnesium	8	2.4

Source: A (Bearden and Fuquay, 1980) and B (Setchell, 1977)

2.4 Semen Collection

Artificial vagina was the most common used method of semen collection in farm animals (Sundarman *et al.*, 2007; Ax *et al.*, 2000). The water jacket inside the artificial vagina was filled to a temperature and pressure preferred by animal species. According to Julie Marzlinda *et al.* (2009), the inside temperature of the artificial vagina should be between 42 to 48°C prior to semen collection for rams in which differ in collecting semen from bull that require the inside temperature of artificial vagina i.e. 50 to 55°C (Ramakrishnan, 2005).

Other method used for semen collection was electro-ejaculation. It was used for animal that were not trained with artificial vagina, suffered from injury, and when conditions were not conducive for normal mounting and ejaculation (Rattray, 1977). Although electro-ejaculation able to collect higher semen volume compared to artificial vagina (Ax *et al.*, 2000), the concentration of the collected semen was lower compared when using artificial vagina. Ramakrishnan (2005) stated that semen collected using electro-ejaculation were rarely as good quality as those that were collected using artificial vagina. Contrast to that, Rattray (1977) reported to have very good result using electro-ejaculation in rams and bulls.

One of the earlier study reported that Equidame phantom was another method to be used in semen collection in which resulted in better hygienic quality and higher sperm concentration compared to the used of conventional artificial vagina (Gordon, 2004). Other less popular method used in semen collection was "gloved hand" technique. It was reported to have significantly more sperm per ejaculate from boar

using glove on bare hand and semen were collected by grasped the penis of the ejaculating male (Rattray, 1977).

2.4.1 Semen Volume

Based on previous study, Dorper ram semen volumes ranged from 0.9 to 1.7 ml (Julie Marzlinda *et al.*, 2009), 0.5 to 2.0 ml in mature ram and 0.5 to 0.7 ml in young ram (Ax *et al.*, 2000), 0.5 to 1.8 ml (Anon, 2008) and 1.0 ml (Bearden and Fuquay, 1980). It is important to immediately place the ejaculated semen samples into a water bath (35°C) and assessed the semen quality (Paulenz *et al.*, 2005).

2.4.2 Semen Colour

Semen colour was determined by the concentration of spermatozoa and partly affected by other factor i.e. the presence of pus cells. According to Ramakrishnan (2005), semen color was estimated through visual appraisal (0=clear, 1=opaque, 2=cloudy white, 3=milky, 4=creamy) which in contrast to the estimation reported by Ax *et al.* (2000) as shown in table 2.4. Ax *et al.* (2000) reported that the semen colour of ram usually milky-white or pale creamy.

Table 2.4 Concentration of ram semen assessed for consistency

Score	Consistency	Number of sperm (x 10^{9})	
		Mean	Range
5	Thick creamy	5.0	4.5 – 6.0
4	Creamy	4.0	3.5 – 4.5
3	Thin creamy	3.0	2.5 – 3.5
2	Milky	2.0	1.0 – 2.5
1	Cloudy	0.7	0.3 – 1.0
0	Watery	Insignificant	

Source: (Ax *et al.*, 2000)

Gordon (2004) stated that the production of semen volume by animals varied accordingly to the species i.e. stallions and boars ejaculate high volume and low density semen while bulls and rams ejaculate low volume and high density semen. The semen production in rams might also be affected by other factors such as technician, time of collection, and collection frequency.

2.5 Extender

When one was evaluating semen for storage or insemination, analysis should be based on the quality of the raw ejaculate, dilution rate and rate of cooling, storage temperature, storage time, container and type of extender used. The main principle of extender was to artificially prolong the spermatozoa life by stabilized the enzymes and protecting sperms cells from unfavourable environment.

The condition that should had in any type of extender were osmotic pressure isotonic with spermatozoa, provide a proper balance of minerals essential to the life of sperm cells, provide an energy source for sperm metabolism, protect sperm cells against cold shock, provide buffering capacity against metabolic products and be free of and protect sperm from bacterial or infectious organism which might prove harmful (Gomes, 1977). There were several types of extender that vary on functions such as for use with fresh semen at farm, cooling and freezing purposes.

Previous study had stated that sperm fertility can be maintained up to six days when suspended in extender and preserved fertility of sperm that stored at 25°C for at least three days (Gomes, 1977). Most semen used for insemination prior to cryopreservation was maintained at chilled temperatures of 4 to 5°C to reduce metabolic rates and prolong sperm fertility. Egg yolk has been used as extender alone or combination with sugars or glycerol in which provide protection against cold shock by yolk lipoproteins and lecithin while fructose for metabolic energy (Gomes, 1977).

2.6 Factors That Influence Reproductive Performance of Ram

2.6.1 Nutrition

According to Fourie *et al.* (2004), intensive feeding practices do enhanced animal's body condition and conformation but have not been fully investigated on how does the practices affected ram's reproductive performance. Ramakrishnan (2005) reported that semen quality in bulls is greatly influenced by nutrition and was supported by Fourie *et al.* (2004) that stated rams fed with high-energy diets had larger testes and high volume of seminal vesicles than the rams that fed with low-energy diets. However, high-energy diets result in low quality seminal characteristics that might cause of

excessive fat deposition on scrotum and adversely affected the scrotal and testicular thermoregulation mechanisms.

Moreover, bulls on high protein ration produced higher semen volume, sperm motility and sperm concentration than bulls fed on low protein ration (Ramakrishnan, 2005). Severe under nutrition and vitamin A deficiencies were the two common causes of impaired reproductive capacity in the male. Prolonged under nutrition might led to a depression or cessation of spermatogenesis and contributed to the decreased of testes size and adversely affected the semen quality (Rattray, 1977). Supplements of vitamin A and C improved sperm production, semen quality and conception rates in swine, while sheep and cattle responded to trace elements such as Copper (Cu), Cobalt (Co), Zinc (Zn), Manganese (Mn), and Iodine (I).

2.6.2 Libido

Libido can be defined as the willingness and eagerness of a male animal to mount and to attempt service of a female. Based on Ramakrishnan (2005), there were several factors affecting libido and mating behavior of bulls including genetics, type of breed, hormonal influences, post-weaning management, nutrition, seasons, environmental temperature and frequency of ejaculation. High frequent collection of semen in particular bulls tended to reduce their libido (Ramakrishnan, 2005). Yet, libido in rams can be intense by feeding high protein supplement (Fourie *et al.*, 2004).

The management of males prior to semen collection was important in maintaining the sex drive of male as it might influence the quality of ejaculation (Gordon, 2004). Providing submaintenance rations in rams have led to depress libido within five to ten weeks and the decline progressed as underfeeding continued. Whereas, overfeeding rams especially that were reared under hot temperature tended to lose libido (Rattray, 1977). Based on Kheradmand and Babaei (2006), during the semen collection, when both the ewe and ram are of the same breed, the reaction time becomes significantly lower but the number of mounts and semen characteristics of volume, sperm concentration and viability did not differ.

2.6.3 Environment

Subtropical and tropical climates might limit reproductive ability. Extremely hot and cold environmental temperature was detrimental to sperm production. The increase of scrotal temperature up to 41°C for three hours in ram causes rapid destruction of pachytene spermatocytes (Gordon, 2004). Rapid changes in temperature induced irreversible anatomical and physiological damage of the sperm. It was important to place collected semen at appropriate thermal environment after collection in order to maintain the sperm metabolism and cellular structure.

2.6.4 Body and Reproductive System Conformation

Volume of semen on every ejaculation varied due to the production of male accessory gland, and it is differ based on individual ability (Zamri, 2008). Measurements of scrotal circumference, testis length, or testis width provide reliable guide to sperm production capacity in male (Ugwu, 2009). Other factors that might also affected semen production were nutrition (Ogbuewe *et al.*, 2009), body weight and testicular morphology (Keith *et al.*, 2009), temperature and humidity (Garcia-Tomas *et al.*, 2008), seasonal variation (Carluccio *et al.*, 2007), social environment (Leboeuf *et al.*, 2007) and raised area (Oyeyemi and Ubiogoro, 2005).

Higher scrotal circumference and scrotal thickness have been recorded in Dorper sheep under similar intensive management conditions compared to extensive management conditions (Almeida *et al.*, 2007). There was a close relationship between testicular size and sperm production in which rams with small testes might not produce enough sperm throughout the joining period to maintain good fertilization rates (Seaman, 2004). Small scrotal circumference incidence happened due to number of factors including immaturity, poor nutrition and developmental defects (Seaman, 2004). Table 2.5 showed the benchmark in interpreted data collected from Breeding Soundness Examination (BSE) on classification of rams at semen evaluation.

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