

# **UNIVERSITI PUTRA MALAYSIA**

EFFECTS OF DIFFERENT ESTRUS SYNCHRONIZATION PROTOCOLS ON REPRODUCTIVE EFFICIENCY OF INDIGENOUS DAMMAR EWES IN YEMEN

NASSER SALEH OBAD HEZAM

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By

NASSER SALEH OBAD HEZAM

Thesis Submitted to the School of Graduate Studies, Universiti Putra Malaysia, in Fulfilment of the Requirement for the Degree of Doctor of Philosophy

November 2012

## DEDICATION

This thesis is exclusively dedicated to my mother, wife, children, brothers, brother-inlaw and all the people of Yemen.



Abstract of thesis presented to the Senate of Universiti Putra Malaysia in fulfilment of the requirement for the degree of Doctor of Philosophy

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November 2012

Chairman: Professor Abd Wahid Haron, PhD

**Faculty: Veterinary Medicine** 

The aim of this study was to compare the effect of different estrus synchronization protocols for long (12 days) and short-term (6 days) on the reproductive efficiency of Dammar ewes during summer and winter in Yemen. A total of 85 ewes were randomly allocated and divided into two experimental groups. The first experiments were conducted for 12 days on those in the long- term (LT) in summer and on those in long-term (LTa) in winter. Similarly, the second experiments were conducted for 6 days on those in short- term (ST) in summer and on those in short- term (STa), in winter. The ewes in (summer, LT) were divided into three subgroups rarely LT1, LT2, and LT3, and

in (winter, LTa) were divided into three subgroups rarely LTa1, LTa2 and LTa3 based on eCG 300 IU injecting which were before 48 h, at CIDR removal and control for LT and LTa groups respectively. Whereas the ewes in (summer, ST) group were divided into two subgroups rarely ST1 and ST2. In addition, in winter (STa) group was divided into two subgroups (STa1 and STa2) based on eCG 300 IU injecting which were at CIDR removal and control for ST and STa respectively. The results showed that onset of estrus in groups LT and LTa for 12 day during summer and winter were shorter than the results in groups ST and STa for 6 day, during summer and winter. The results of duration of estrus showed in LT1, LTa1, LT2, LTa2, and ST2 were shorter than the results in STa1, ST1, LT3, STa2 and LTa3. However, the results of cessation of estrus in LTa1, LT1, LT2, LTa2 and LT3 were shorter than the results in those of LTa3, STa1, ST2, STa2 and ST1 there were significant difference (P<0.05) between the 12 day and the 6 day during summer. The result of estrus response, for pregnant and lambing percentages in ewes was 100% in both 12 day and 6 day groups during summer and winter. However, there was no significant difference (P>0.05) between 12 day and 6 day during summer and winter. The litter size in LT1, LT2, LTa2 and LTa1 were higher than in STa1, STa2, ST1, ST2, LT3 and LTa3 there was significant difference (P<0.05) between 12 day and 6 day during summer and winter. The fecundity rates, in LT1, LT2, LTa1 and LTa2 were higher than in the ST2, STa1, ST1, STa2, LT3 and LTa3 there was significant difference (P<0.05) between 12 day and 6 day during summer and winter. However, the twining rates in LT3, LT1, LT2, LTa1 and LTa2 were higher than in LTa3, STa1, STa2, ST1 and ST2 there was significant difference (P<0.05) between 12 day and 6 day during summer and winter. The result of this study indicated that the time of eCG injection had an effect on the litter size but had no effect on the estrus response,

pregnancy, lambing, during summer and winter. In conclusion, the results of the present study showed that the short-term (6 days), progesterone treatment in Dammar ewes had a similar effect with the long-term (12 days) to estrus synchronization, pregnancy and lambing rates during summer and winter. Moreover, the litter size in ewes treated by eCG was higher than ewes that were not treated with eCG in both the 12 day and 6 day groups during summer and winter.



Abstrak tesis yang dikemukakan kepada Senat Universiti Putra Malaysia sebagai memenuhi keperluan untuk ijazah Doktor Falsafah

## KESAN PENYEGERAKAN ESTRUS BERLAINAN PROTOKOL ON KECEKAPAN PEMBIAKAN DARI BETINA DAMMAR PRIBUMI DI YAMAN

Oleh

#### NASSER SALEH OBAD HEZAM



Pengerusi: Profesor Abd Wahid Haron, PhD

Fakulti: Perubatan Veterinar

Tujuan kajian ini adalah untuk membandingkan kesan protokol penyegerakan estrus berbeza untuk tempoh yang lama (12 hari) dan jangka pendek (6 hari) pada kecekapan pembiakan Dammar betina semasa musim panas dan musim sejuk di Yaman. Sebanyak 85 ekor kambing betina secara rawak diperuntukkan dan dibahagikan kepada dua kumpulan eksperimen. Eksperimen pertama telah dijalankan selama 12 hari ke atas mereka dalam jangka panjang (LT) pada musim panas dan pada mereka yang dalam jangka panjang (LTA) pada musim sejuk. Begitu juga, eksperimen kedua telah dijalankan selama 6 hari kepada orang-orang dalam jangka pendek (ST) pada musim panas dan pada mereka dalam jangka kepada tiga kumpulan kecil jarang LT1, LT2, dan LT3, dan dalam (musim sejuk, LTA) telah dibahagikan kepada tiga subkumpulan jarang LTa1, LTa2 dan LTa3 berdasarkan IU ECG 300 suntikan yang sebelum 48 h , pada

penyingkiran CIDR dan kawalan untuk LT dan kumpulan LTA masing-masing. Manakala betina dalam kumpulan (musim panas, ST) telah dibahagikan kepada dua subkumpulan jarang ST1 dan ST2. Di samping itu, pada musim sejuk (STA) kumpulan telah dibahagikan kepada dua subkumpulan (STa1 dan STa2) berdasarkan IU ECG 300 suntikan yang masing-masing berada di CIDR penyingkiran dan kawalan untuk ST dan Sta. Hasil kajian menunjukkan bahawa bermulanya estrus dalam kumpulan LT dan LTA selama 12 hari semasa musim panas dan musim sejuk adalah lebih pendek daripada keputusan dalam kumpulan ST dan STA selama 6 hari, semasa musim panas dan musim sejuk. Keputusan tempoh estrus menunjukkan di LT1, LTa1, LT2, LTa2, dan ST2 adalah lebih pendek daripada keputusan dalam STa1, ST1, LT3, STa2 dan LTa3. Walau bagaimanapun, keputusan pemberhentian estrus pada LTa1, LT1, LT2, LTa2 dan LT3 adalah lebih pendek daripada keputusan dalam mereka LTa3, STa1, ST2, STa2 dan ST1 terdapat perbezaan yang signifikan (P <0.05) antara hari ke 12 dan 6 hari semasa musim panas. Hasil tindak balas estrus, peratusan mengandung dan beranak dalam betina adalah 100% dalam 12 siang dan 6 kumpulan hari semasa musim panas dan musim sejuk. Walau bagaimanapun, tidak terdapat perbezaan yang signifikan (P > 0.05) antara hari 12 dan 6 hari semasa musim panas dan musim sejuk. Saiz sampah di LT1, LT2, LTa2 dan LTa1 adalah lebih tinggi daripada pada STa1, STa2, ST1, ST2, LT3 dan LTa3 terdapat perbezaan yang signifikan (P <0.05) di antara 12 hari dan 6 hari semasa musim panas dan musim sejuk. Kadar kesuburan, LT1, LT2, LTa1 dan LTa2 adalah lebih tinggi daripada di ST2, STa1, ST1, STa2, LT3 dan LTa3 terdapat perbezaan yang signifikan (P <0.05) di antara 12 hari dan 6 hari semasa musim panas dan musim sejuk. Walau bagaimanapun, kadar Twining pada LT3, LT1, LT2, LTa1 dan LTa2 tinggi daripada di LTa3, STa1, STa2, ST1 dan ST2 terdapat perbezaan yang signifikan (P < 0.05) di antara

12 hari dan 6 hari semasa musim panas dan musim sejuk. Hasil kajian ini menunjukkan bahawa masa ECG suntikan mempunyai kesan kepada saiz sampah tetapi tidak mempunyai kesan ke atas sambutan estrus, mengandung, beranak, semasa musim panas dan musim sejuk. Kesimpulannya, hasil kajian ini menunjukkan bahawa jangka pendek (6 hari), progesteron rawatan dalam betina Dammar mempunyai kesan yang sama dengan jangka panjang (12 hari) untuk penyegerakan estrus, mengandung dan beranak kadar semasa musim panas dan musim sejuk . Selain itu, saiz sampah di betina dirawat oleh ECG adalah lebih tinggi daripada betina yang tidak dirawat dengan ECG dalam kedua-dua hari 12 dan 6 kumpulan hari semasa musim panas dan musim sejuk.

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#### APPROVAL

I certify that an Examination Committee has met on 27 November 2012 conduct the final Examination of Nasser Saleh Obad Hezam on his degree thesis entitled "Effects of different estrus synchronization protocols on reproductive efficiency of indigenous Dammar ewes in yemen" in accordance with Universiti Pertanian Malaysia (Higher Degree) Act 1980 and Universiti Pertanian Malaysia (Higher Degree) Regulations 1981. The Committee recommends that the student be awarded the Doctor of Philosophy.

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## DECLARATION

I declare that the thesis is my original work except for quotations and citations, which have been duly acknowledged. I also declare that it has not been previously, and is not concurrently, submitted for any other degree at Universiti Putra Malaysia or at any other institution.



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# LIST OF ABBREVIATIONS

AI	Artificial insemination
AV	Artificial vagina
BCS	Body condition score
BSE	Breeding soundness examination
BW	Body weight
CIDR	Controlled internal drug release device
CIDR-G	Controlled internal drug release device for goat
CL	Corpus luteum
CR	Conception rate
DE	Duration of estrus
eCG	Equine chorionic gonadotrophin
EE	Electro-ejaculation
EIA	Enzyme immunoassay
ET	Embryo transfer
FGA	Fluorogestone acetate
FSH	Follicle-stimulating hormone
g	Gram
GnRH	Gonadotropin-releasing hormone
GPS	Geographic position system
hCG	Human Chorionic Gonadotrophine
IM	Intramuscular

	IU	International unit
	LAI	Laparoscopic insemination
	LH	Luteinizing hormone
	LT	Long- term (12 days in summer)
	LT1	Group CIDR + eCG before 48h CIDR removal
	LT2	Group CIDR + eCG at CIDR removal
	LT3	Group CIDR control without eCG
	LTa	Long- term (12 days in winter)
	mg	Milligram
	μg	Microgram
	MGA	Melengesterol acetate
	MHz	Megahertz
	NaCl	Sodium chloride
	ng	Nano gram
	NRR	Non-return estrus rates
	ES	Estrus synchronization
	P4	Progesterone
	PG	Progestogen gonadotropins
	PGF2a	Prostaglandin F2α analogue
	PMSG	Pregnancy mare serum gonadotrophine
	PR	Pregnancy rate
	RIA	Radioimmunoassay
	SC	Scrotal circumference

SEM	Standard error of the mean
SMB	Synchro-mate-B
Spp	Species
SPSS	Statistical package for the social sciences
ST	Short- term (6 days in summer)
ST1	Group CIDR + eCG at CIDR removal
ST2	Group CIDR control without eCG
STa	Short- term (6 days in winter)
TAI	Trans-cervical insemination
TL	Testicular length

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#### **CHAPTER 1**

#### **INTRODUCTION**

The republic of Yemen is at the south western corner of the Arabian Peninsula, between 12° and 17.7° north and 43.5° to 52° easts. It includes many islands, Socotra (the largest in the Arabian Sea) and Kamaran in (the Red Sea). The country to the north is enclosed by Saudi Arabia, to the east Oman, to the south Arabian sea and the west the Red sea. The land area is about 555,000 km<sup>2</sup> (Alabsi, 2001). The population of Yemen was 24,771,809 (Population May2012 Country Ranks, By Rank).

Yemen has a semi-arid to arid climate and the rainy seasons are in spring (March to May) and summer (July – September). Its influence is most noticeable at higher altitudes in the west of the country. The mountain ranges rise from sea level to over 3,600 m within 100 km from the Red Sea. Rainfall rises from less than 50 mm along the Red Sea and Gulf of Aden coasts to a maximum of 500-800 mm in the western Highlands and decreases steadily to below 50 mm inland (Alabsi, 2001).

Average temperatures decrease more or less linearly with altitude. The rise of the air masses over the mountains provides a cooling mechanism, which stimulates the rainfall. Average temperatures are dominantly controlled by altitude. There is an approximate linear relationship, with an average temperature gradient of about 0.65<sup>o</sup>C per 100m difference in elevation. At lower altitudes (below 500 m) in coastal areas, this relationship is disturbed by the moderating effect of the sea (Alabsi, 2001).

The temperatures, the eastern plains and western featuring temperatures as high as in summer to  $42^{\circ}$ C and landing in the winter to  $25^{\circ}$ C and temperatures drop gradually towards the highlands due to the factor so high temperatures reach  $33^{\circ}$ C maximum and 20 °C minimum in winter the degrees minimum temperature on the highlands to nearly zero degrees and -  $12^{\circ}$ C in Dammar (Alabsi, 2001). The range of temperatures in Dammar is between  $11^{\circ}$ C-  $13^{\circ}$ C in winter and between  $16^{\circ}$ C-19 °C in summer (Alabsi, 2001).

The humidity is high in the coastal plains up to more than 80% while landing toward the inside, bringing the minimum rate in the desert areas and that the percentage of humidity of 15% also characterized by the Directorate of and higher province moderate Dhamar atmosphere and breathtaking nature and agricultural runways at the top of the magnificence and beauty (Alabsi, 2001). The humidity in

Dammar is between 20-40% during June, September and February and 40-60% during May, July and September (Alabsi, 2001).

Accurding to Yemenian farmers that there is no spring season in Yemen (Personal communication, 2010), but a study by Kessler (1989) mentioned the season of spring and showed that the annual rainfall is concentrated during two periods of the year, namely spring (March to April) and summer (May and August). The average annual temperature is 15<sup>o</sup>C during the long and dry winter season (November to February) and frosts are common. Sheep are of fleeced (hairy) type and mainly kept for meat which is a highly valued product (Kessler, 1989).

Summer in Yemen is from May to August with longer days and shorter nights. The length of day ranges from 12.9 to 14hours and the length of the night is between 10 and 11.1 hours. This season is similar to the non breeding season in Egypt. The four months in summer yield a good growth of grass. Nevertheless, the winter months from November to February have longer nights and shorter days. The length of days for the winter ranges from 11.1 to 11.7 hours and the nights range from 12.3 to 12.9 hours but this winter is defined as dry season, because it does not generate good growth of grass. Moreover this season is considered the same as the breeding season (Personal communication, 2010)

Although the transition from summer to winter is two months which includes September and October, they are of equal hours between the length of the days and the length of the nights. This season is considered the same as transitional from the non-breeding season to a breeding season and it has good growth of grass.

Spring, have March and April and is considered a transitional period from winter to summer it have equal hours between the length of the night and the length of the day. The spring is considered as the transitional period from the breeding season to the non- breeding season and yields a very good growth of grass (Heny, 1981; Hunter and Heath, 1984).

More recent studies showed that the seasons in Yemen are similar to the season in Egypt, (Aboulnga and Aboulela 1985, Marri *et al.*, 2009). In their studies (which were from December to February) they showed that the winter is considered as a breeding season. However spring starts from March until April and autumn starts from May until June whereas the summer which is considered as a non breeding season starts from July until November. By comparison, the length of the days during summer in Egypt is longer than the length of days in Yemen. By contrast, the length of nights during winter in Egypt is longer than the length of night in Yemen.

However, the length of days during summer in Europe is the longest compared to the length of days in Yemen and Egypt. Similarly, the length of nights during winter in Europe is the longest in comparison to the length of night in Yemen and Egyptian. So the breeding and non-breeding seasons in European and Egyptian sheep are very clear than in Yemenian sheep. For this reason the Dammar ewes in Yemen have exhibited estrus throughout the year.

Grassland and woodlands are natural pasture and make up nearly 42.7 % of the land area. This natural vegetation cover comprises a wide spectrum and various species of trees, shrubs, grasses, herbs and annual plants and includes a number of succulents. These plants represent a relevant means of flexibility in climatic fluctuations and if properly managed, can provide sustainable forage resources all the year round (Alabsi, 2001).

The main natural forages namely High Mountains and Highland Plain regions are perennial, indigenous, tall and short grasses such as Andropogon spp., Pennisetum spp., Panicum spp., Themeda triandra, Dichanthium spp., and Eragrostis spp. Very desirable perennial and annual legumes are parts of the vegetation in these regions such as Medicago sativa, Medicago lupulina and Medicago hispida (these latter two lucernes are usually part of the wild pasture flora) Trifolium spp., and Indigofera spp (Alabsi,2001).

Synchronization of the estrus cycle is a technique, which is used to bring a large number of animals in a flock into overt heat at the predetermined time (Hutchens, 2005; Panhwar, 2007; Wildeus, 2007). The technique offers an opportunity to increase the efficiency of animal production in different ways. Estrus synchronization (ES) permits kidding and lambing to occur at suitable times in order to take advantage of feed and labour, availabilities, markets, and increasing price trends (Panhwar, 2007; Wildeus, 2007).

Estrus synchronization in goats and sheep is achieved by the control of the luteal phase of the estrus cycle, either by providing exogenous progesterone or by inducing premature luteolysis. The latter approach is not applicable during seasonal anestrus, whereas exogenous progesterone in combination with gonadotropin can be used to induce and synchronize estrus in anovular does and ewes (Wildeus, 1999).

Progestagen analogues have been widely used to induce and synchronize estrus in small ruminants (Emsen and Yaprak, 2006). The various methods of estrus synchronization that have been used in many researches showed that a timed hormonal treatment is the most suitable and successful method to synchronize estrus in goat and sheep in farms (Whitley and Jackson, 2004). The most widely used procedures for synchronization induced estrus are FGA, MAP and CIDR with an intramuscular injection of PMSG or eCG at progestagen withdrawal (Romano, 2002).

Estrus response and fertility vary greatly when the intra-vaginal sponge is applied depending on the species, breed, co-treatment, management, breeding season and mating system (Dogan and Nur, 2006; Emsen and Yaprak, 2006). The successfulness of progestagen treatment is also affected by the body health condition, the physiological state of ewes, and breeding or artificial insemination (Menchaca and Rubianes, 2004).

The long-term progestagen treatments from 12 to 14-days are widely used in small ruminants to induce and synchronize estrus and as a result a high percentage of the treatment in animals showed estrus but the fertility rate is lower than that natural estrus (Robinson *et al.*, 1970). Other researches in ewes and does showed that short-term treatments of 5 to 6 days with different progestagen devices during the non-breeding season were as effective as long-term treatment to induce estrus and the subsequent fertility was also high (Rubianes *et al.*, 1998). However, the application of a long from treatment with progesterone also induced estrus in a high proportion of seasonal anestrus ewes but the subsequent pregnancy rate was low (Rubianes *et al.*, 1998). Recently, Ungerfeld and Rubianes (1999a) found that short-term treatment (5- 6 days) with different progestagen devices during the non-breeding season was as effective as long-term treatment to induce estrus and high proportion of seasonal anestrus ewes but the subsequent pregnancy rate was low (Rubianes *et al.*, 1998). Recently, Ungerfeld and Rubianes (1999a) found that short-term treatment (5- 6 days) with different progestagen devices during the non-breeding season was as effective as long-term treatment to induce estrus and the subsequent fertility.

During the breeding season and with natural mating, Vinoles *et al.* (2001) obtained a higher pregnancy rate (87%) in ewes after a short-term treatment (6 days of medroxyprogesterone acetate (MAP) sponges compared to the traditional 12-day treatment either with (67%) or without (63%) an equine chorionic gonadotropin (eCG) dose at the time of sponge withdrawal. Various studies evaluated different PMSG dose levels, injection times and alternative types of gonadotropins (Zeleke *et al.*, 2005). However, some studies maintained that the effect of PMSG treatment on fertility is still controversial (Drion *et al.*, 2001).

Intra-vaginal devices which contained different types of progestagens and were maintained during 6-14 days associated with or without PMSG or PMSG and Prostaglandin PGF $\alpha$ 2 combination were used (Ataman *et al.*, 2006). As a result of the estrus synchronization protocols, a high percentage of ewes showed estrus whereas fertility was lower than in ewes showing natural estrus. This low fertility rate has been attributed to changes in the hormonal milieu that results in corresponding between estrus and ovulation (Scaramuzzi *et al.*, 1988), with a subsequent alteration in sperm transport (Pearce and Robinnson, 1985).

Progestagen 5-day treatment was used in combination with PGF $\alpha$ 2 to improve estrus synchronization with good results but the potential benefit of a short-term treatment alone in combination with fixed time artificial insemination has not yet been evaluated (Menchaca and Rubianes, 2004). Recent studies have focused on the duration of the progestagen-based synchrony treatments (Ataman *et al.*, 2006). Indigenous sheep are more important in Yemen people for meat consumption than other animals such as cattle and camel. But the numbers of sheep is still small. Though different methods of estrus synchronization have been investigated worldwide, these methods have not yet been performed in Yemen. Estrus synchronization is influenced by breed, nutrition, season, device of progesterone (CIDR or sponge), the dosage of eCG, time of injection of gonadotropins, progestagen treatment protocols, presence of the male after progesterone removal, co-treatment, management, and mating system (Amer, and Hazzaa, 2009). Season is one of the important factors affecting reproduction in small ruminants (Devendra and Burns, 1970). So the present study on the effects of different estrus synchronization protocols on the reproductive efficiency of Dammar ewes in Yemen during winter (dry season) and summer (warm season). In order to achieve this goal, the progesterone analogue is usually used to synchronize estrus in sheep.

The farmers in Yemen are only using the traditional method of breeding animals without any help to improve their animals. The farmers depend mainly on using only natural estrus as they lacked the knowledge of the artificial estrus synchronizations and none has yet to make use of the method of estrus synchronization in Yemen in order to improve their farm animals.

### **1.1 Problem statement**

Some researchers evaluated the effect of long and short progestagen sponge treatment for estrus synchronization with one time injection of eCG technique in breeding and out breeding season in some ewe breed for studies done in onset and estrus response. In addition, no researcher has investigated the effect of different times of injection of eCG to study the fertility and estrus in Dammar indigenous sheep in Yemen. There is no information regarding the estrus synchronization efficiency and fertility which was induced by hormonal treatment of indigenous goats, sheep and others animal farms during summer and winter in Yemen. The present study was conducted to determine the effects of different techniques of synchronization, short and long protocol for sheep during summer and winter. The results from this study was used to compare the results with natural estrus which the farmers may have some encouragement to perform the synchronization themselves and we will be able to introduce the techniques of synchronizations to farm animals in Yemen. The objectives of this study are followed below.

#### 1.2 The objectives of the study

1. To collect some general information on sheep feeding in Yemen that was includes the sheep population, sheep breeds, and husbandry practices as well as the breeding performance from estrus behavior, natural mating and the lambing rates.

2. To compare the (onset of estrous, duration of estrous, estrus cessation, estrus response, pregnancy, lambing rate litter size) in Dammar ewes with long term (12 days) of synchronization oestrus protocols with different injection of eCG during summer.

3. To compare the (onset of estrous, duration of estrous, estrus cessation, estrus response, pregnancy, lambing rate litter size) in Dammar ewes with short term (6 days) of synchronization oestrus protocols with different injection of eCG during summer.

4. To compare the (onset of estrous, duration of estrous, estrus cessation, estrus response, pregnancy, lambing rate litter size) in Dammar ewes with long term (12

days) of synchronization oestrus protocols with different injection of eCG during winter.

5. To compare the (onset of estrous, duration of estrous, estrus cessation, estrus response, pregnancy, lambing rate litter size) in Dammar ewes with short term (6 days) of synchronization oestrus protocols with different injection of eCG during winter.



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