UNIVERSITI PUTRA MALAYSIA

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FPV 2011 18

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By

MUHAMMAD MODU BUKAR

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

December 2011

DEDICATION

In the name of Allah, the Beneficient, and the Merciful. This thesis is dedicated to my precious mother, Hajja Kaltume Mustapha who nurtured me with unconditional love. The thesis is also dedicated to my father, Alhaji Bukar Madu for his unflinching support and encouragement all these years, to all my siblings for their confidence in me; to my wife, Yagana Baba Mele for her support and finally, to my children, Aisha, Kaltume, Abubakar, Idris and Maryam.

Abstract of the thesis presented to the Senate of Universiti Putra Malaysia in fulfillment of the requirement for the degree of Doctor of Philosopy

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December 2011

Chairman: Associate Professor Rosnina Yusoff, PhD

Faculty: Veterinary Medicine

At present, most of the information on the pattern of antral follicular development on ruminants were gathered from studies conducted in cattle and sheep. However, very little amount of literature exists for goats, especially the non-seasonal polyoestrous goats domesticated in the humid tropics. Thus, the focus of this thesis was to examine the effects of oestrus synchronisation with $PGF_{2\alpha}$ and P_4, alone or in combination, with and without gonadotrophins on antral follicular development in goats. In the first experiment, the effects of oestrus synchronisation with $PGF_{2\alpha}$ and CIDR on follicular

development, plasma IGF-1 concentration and its association with follicle population were studied.

Twenty-four Boer x Australian feral crossbred goats that were between 3-4 years of age, with a mean bodyweight of 35.0 ± 2.7 kg and a median body condition score of 3 were used in this study. The goats were equally divided into 3 groups: $PGF_{2\alpha}$ (A), CIDR (B) and naturally cycling (C). Group A was synchronised with two intramuscular injections of 125 µg cloprostenol, 11 days apart while Group B was synchronised with CIDR inserted in the vagina for 17 days. Group C was not oestrus synchronised. Three waves of follicular development were most frequently observed (58%), followed by 4 waves (31.6%). There were no significant differences ($P>0.05$) between treatment groups for the other parameters associated with follicular development. A low positive correlation ($r=0.14$) was observed between IGF-1 concentration and the number of 3 mm follicles and between IGF-I concentration and mean number of follicles ($r=0.13$). The low correlation between IGF-1 concentration and follicular population suggests a weak association between plasma IGF-1 concentration and follicular development in goats.

The objective of the second experiment was to analyse the effects of $PGF_{2\alpha}$ and P_4 methods of oestrus synchronisation with or without eCG and FSH on oestrus response and ovulation rates in the goats. The serum cortisol concentrations were also measured to determine if stress occurred during handling for ultrasonography, in the goats raised

under a hot and humid tropical environment. There were 9 groups of goats and each group was synchronised with different protocol using $PGF_{2\alpha}$, FGA and their combinations and either 5 mg FSH or 300 IU eCG. All the synchronised goats that were given eCG exhibited oestrus (100%). However, the number of follicles was higher ($P< 0.05$) in FSH synchronised groups than the eCG synchronised groups. It was concluded that the $PGF_{2\alpha}$ + FGA + FSH method of oestrus synchronisation is the most promising alternative to oestrus synchronisation with eCG in goats. The serum cortisol concentrations were not significantly different ($P< 0.05$) between goats handled for ultrasonography and the control group. The third experiment was conducted to examine the effects of eCG on preovulatory follicle growth and time of ovulation. The number of follicles, maximum size of the ovulatory follicles and time of ovulation in a total of fifty-one FGA + $PGF_{2\alpha}$ + eCG synchronised goats and those without eCG were determined. It was found that eCG significantly ($P<0.05$) increased the total follicle number, maximum follicle diameter and reduced the time to ovulation by 20 hrs.

In conclusion, this is the first report of the pattern of follicular development during natural and synchronised oestrous cycles in non-seasonal polyoestrous goats raised in the hot humid tropics. The effects of oestrus synchronisation with prostaglandin $F_{2\alpha}$ and P_4 on follicular development were similar and both hormones increased the number of follicles and IGF-1 concentration in the synchronised and subsequent oestrous cycles compared with the naturally cycling group. Of the FSH-based oestrus synchronisation protocols, the $PGF_{2\alpha}$ + FGA + FSH resulted in higher number of

follicles than any of the eCG-based oestrus synchronisation protocols evaluated and is the most promising alternative to oestrus synchronisation with eCG in goats.

Key words: Ultrasonography, follicular development, oestrus response, IGF-1 concentration, cortisol

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

PERKEMBANGAN FOLIKEL ANTRUM DAN GERAKBALAS OESTRUS PADA KAMBING YANG DISELARASKAN ESTRUS DAN YANG BERKITARAN SEMULA JADI

Oleh

MUHAMMAD MODU BUKAR

Disember 2011

Pengerusi: Profesor Madya Rosnina Yusoff, PhD

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Sehingga kini banyak maklumat mengenai perkembangan folikel antrum pada haiwan ruminan diperolehi daripada kajian yang melibatkan lembu dan biri-biri, dan maklumat mengenainya pada kambing adalah sangat sedikit, terutama bagi kambing poliestrus tidak bermusim yang dibelajinak di tropik melengas panas. Sehubungan itu, fokus tesis ini adalah untuk mengkaji kesan penyelarasan estrus dengan $PGF_{2\alpha}$ dan P_4, bersendirian atau secara kombinasi, dengan dan tanpa gonadotropin terhadap perkembangan folikel antrum. Dalam eksperimen pertama, kesan penyelarasan estrus menggunakan $PGF_{2\alpha}$ dan CIDR terhadap perkembangan folikel, kepekatan plasma IGF-1 dan korelasi dengan populasi folikel dikaji. Dua-puluh empat ekor kambing kacukan Boer x feral Australia yang berumur antara 3 dan 4 tahun, dengan berat badan

35.0 ± 2.7 kg dan skor median 3 bagi keadaan badan digunakan untok kajian ini. Kambing tersebut dibahagi sama kepada 3 kumpulan: $PGF_{2\alpha}$ (A), CIDR (B) dan berkitaran semulajadi/kawalan (C). Kumpulan A diselaraskan dengan dua kali suntikan 125μg cloprostenol melalui intraotot dengan jarak 11 hari, manakala Kumpulan B diselaraskan dengan CIDR yang diletakkan ke dalam faraj selama 17 hari. Kumpulan C tidak diselaraskan estrusnya. Perkembangan folikel 3-gelombang paling kerap dicerap (58%), diikuti oleh 4 gelombang (31.6%). Tiada perbezaan signifikan ($P>0.05$) di antara kumpulan yang dikaji bagi parameter yang berkaitan dengan perkembangan folikel. Suatu korelasi positif rendah ($r = 0.14$) dicerap antara kepekatan IGF-1 dan bilangan 3 mm folikel dan antara kepekatan IGF-I dan purata bilangan folikel ($r = 0.13$). Korelasi yang rendah antara kepekatan IGF-1 dan populasi folikel mencadangkan perhubungan lemah antara kepekatan plasma IGF-1 dan perkembangan folikel pada kambing.

Objektif eksperimen kedua adalah untuk menganalisis kesan kaedah penyelarasan estrus menggunakan $PGF_{2\alpha}$ dan P_4 dengan atau tanpa eCG dan FSH terhadap gerakbalas estrus dan kadar pengovulanan pada kambing. Kepekatan serum kortisol juga disukat untok menentukan jikalau stress berlaku semasa pengendalian untok ultrasonografi di kalangan kambing yang dibelajinak di bawah persekitaran tropik panas dan melengas. Terdapat 9 kumpulan kambing dan setiap kumpulan diselaras dengan protokol yang berbeza menggunakan $PGF_{2\alpha}$, FGA serta kombinasi mereka dan 5 mg FSH atau 300 IU eCG. Semua kambing terselaras dan diberikan eCG mempamerkan oestrus (100%). Walau bagaimanapun, bilangan folikel adalah lebih

tinggi ($P<0.05$) dalam kumpulan FSH daripada kumpulan eCG. Kesimpulannya ialah kaedah $PGF_{2\alpha}$ + FGA + FSH merupakan alternatif harapan terhadap penyelarasan estrus mengguna eCG pada kambing. Kepekatan serum kortisol tidak memberi perbezaan signifikan ($P>0.05$) antara kambing yang dikendalikan untok ultrasonografi dan kumpulan kawalan.

Eksperimen ketiga dijalankan untuk meneliti kesan eCG terhadap pertumbuhan folikel praovulatori dan masa pengovulanan. Bilangan folikel, saiz maksimum folikel ovulatori dan masa pengovulanan pada 51 ekor kambing yang diselaraskan dengan FGA + $PGF_{2\alpha}$ + eCG dan tanpa eCG ditentukan. Didapati bahawa eCG meningkatkan dengan signifikan ($P<0.05$) jumlah bilangan folikel, garispusat folikel maksimum dan mengurangkan masa pengovulanan ovulasi sebanyak 20 jam.

Kesimpulannya, ini merupakan laporan pertama terhadap corak perkembangan folikel semasa kitaran estrus semulajadi dan estrus terselaras di kalangan kambing poliestrus tidak bermusim yang dibelajinak di tropik melengas panas. Kesan penyelarasan estrus menggunakan $PGF_{2\alpha}$ dan P_4 terhadap perkembangan folikel adalah sama, dan kedua-dua hormon telah meningkatkan bilangan folikel dan kepekatan IGF-1 pada kitaran estrus yang diselaraskan dan kitaran estrus berikutnya berbanding kumpulan kitaran semulajadi. Dari protokol penyelarasan estrus berdasarkan FSH, kumpulan $PGF_{2\alpha}$ + FGA + FSH menghasilkan bilangan folikel yang tinggi berbanding penyelarasan estrus berasaskan eCG dan ia merupakan alternatif harapan terhadap penyelarasan estrus menggunakan eCG pada kambing.

ACKNOWLEDGEMENTS

Firstly, in all humility, I wish to extend my profound gratitude to Al-Rahman, the All Knowing, Lord of the worlds for all that I am and have done. Secondly, my most sincere appreciation to the chairperson of my Supervisory Committee, Associate Professor Dr. Rosnina Yusoff for her diligent supervision of my research work and her sincere concern for my welfare. I also wish to express my appreciation to members of my Supervisory Committee; Professor Dr. Mohammed Ariff Omar, Professor Dr. Abd Wahid Haron, Associate Professor Dr. Gurmeet K. Dhaliwal and Associate Professor Dr. Mohammed Azam Khan for their constructive criticisms and support throughout my PhD candidature. I am very grateful to Professors Ariff and Wahid for their concern and purchase of immunoassay kits to enable me to complete my analyses. Dr. Azam's assistance and encouragement during my stay at ar-Raudhah farm is most appreciated.

My most sincere appreciation also goes to Mr. K.C.Yap of the Theriogenology and Cytogenetics unit, for his technical support and advice. I am also grateful to Mr. P. Ganesamurthi, Mohammed Fahmi of Theriogenology and Cytogenetics Unit, and Mr. Mohammed Azri of and Molecular Biology Laboratory, respectively for their technical assistance. It was my privilege to have as my laboratory colleagues, Drs. Nur Husien Yimer, Abdul Malik, Aqeel Memon, Soe Win Naing, Mojgan Nazari, Kajal Sarsaifi, Khumran Mada, and Hadi Hajarian. I would always remember their camaraderie. Other

fellow students at the Faculty of Veterinary Medicine were also very helpful during the course of my studies; Drs Ibrahim Abdulazeez, Ibrahim Anka, Lawan Adamu, Hassan Ismail and Abubakar Mustapha.

I wish to aknowledge the assistance of the staff of ar-Raudhah Biotech farm, Kuang for their assistance. I also wish to thank my friends in Nigeria for their support and encouragement. These friends include Drs. Bukar Goni, Ali Adamu, Maryam Yachilla,. Other friends were Ahmadu Abatcha, Abdullahi Bukar and Mansur Mohammed.

I thank my Professors, Abdullahi Ribadu, Jibrilla Amin, Abdullahi Mohammed and Abdullahi Mani who encouraged me to venture out to further my studies. I value their confidence and career advice. My appreciation also goes to my senior colleagues, Prof. U.K. Sandabe, Drs M. A. Waziri, H. Kumshe, U. Tijjani, Y. Geidam, Umar Isa and D. El yuguda who constantly sent me words of encouragement. I wish to thank Universiti Putra Malaysia for providing me the opportunity to undergo the PhD programme and for the graduate research fellowship which facilitated my research. Finally, I wish to express my appreciation to University of Maiduguri, Nigeria for granting me the study leave to pursue my PhD at the Faculty of Veterinary Medicine, Universiti Putra Malaysia.

I certify that a Thesis Examination Committee has met on 16th December 2011 to conduct the final examination of Muhammad Modu Bukar on his thesis entitled "Antral Follicular Development and Oestrous Response in Oestrous Synchronized and Naturally Cycling Goats" in accordance with the Universities and University Colleges Act 1971 and the Constitution of the Universiti Putra Malaysia [P.U.(A) 106] 15 March 1998. The Committee recommends that the student be awarded the Doctor of Philosophy.

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This thesis was submitted to the Senate of Universiti Putra Malaysia and has been accepted as fulfilment of the requirement for the degree of Doctor of Philosophy. The members of the Supervisory Committee were as follows:

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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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MUHAMMAD MODU BUKAR

Date: 16th December 2011

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LIST OF ABBREVIATIONS

ACTH	adrenocorticotrophic hormone
AI	artificial insemination
ANOVA	analysis of variance
BCS	body condition score
CIDR	controlled internal drug release
CL	corpus luteum
DF	dominant follicle
eCG	equine chorionic gonadotrophin
EIA	enzyme immunoassay
ELISA	enzyme-linked immunosorbent assay
FGA	flugestone acetate
FAOSTAT	food and agriculture organization of the united nations
FSH	follicle stimulating hormone
g	gravity
GnRH	gonadotropin releasing hormone
HPA	hypothalamic-pituitary axis
^{125}I	Iodine 125
IGFBP	insulin-like growth factor binding protein

IGF-1	insulin-like growth factor 1
IGF-2	insulin-like growth factor 2
IGFR-1	insulin-like growth factor receptor 1
IGFR-2	insulin-like growth factor receptor 2
IU	international unit
kDa	kilodalton
LH	luteinizing hormone
MAP	medroxyprogesterone acetate
MHz	megahertz
P_4	progesterone
P450scc	cytochrome P450 side chain cleavage
PBS	phosphate buffer saline
$PGF_{2\alpha}$	protaglandin F2 alpha
RIA	radioimmunoassay
SEM	standard error of mean

CHAPTER 1

GENERAL INTRODUCTION

In 2009, the world goat population was estimated at 879 million (Dubeuf and Boyazoglu, 2009; FAOSTAT, 2011). Out of this total, the largest population is found in Asia (524 million), followed by Africa (298 million). When these two populations are combined, they make up 94 % of the world goat population (FAOSTAT, 2011). The world-wide appeal of goats is due to their small manageable size, prolificacy, superior adaptive characteristics and their ability to thrive in adverse conditions and limited feed resources of arid, semi arid or tropical environment (Silanikove, 2000a; Lebbie, 2004). Overall goat productivity depends on genotype, environment and husbandry factors, thus the intensive management system is used to mitigate the negative effects of these factors in order to improve the efficiency of goat reproduction (Alexandre and Mandonnet, 2005).

In tropical countries like Indonesia and Malaysia, goats breed throughout the year from January to December (Devendra and Burns, 1983; Sodiq et al., 2003). This provides an opportunity for a greater control of their reproductive cycle to exploit the naturally high prolificay of the goats. Manipulation of the oestrous cycle through oestrus synchronisation is an effective tool to improve reproduction in food animals (Greyling, 2010). Exogenous hormones are used to control the oestrous cycle so that a large number of females exhibit oestrus behaviour within a short period of time. In goats, the treatments used for oestrus synchronisation include the

administration of prostaglandin $F_{2\alpha}$ ($PGF_{2\alpha}$), or progesterone (P_4) and their combination (Adams et al., 1992a; Wildeus, 2000; Whitley and Jackson, 2004).

In earlier studies, oestrus synchronisation gave better results when $PGF_{2\alpha}$ or P_4 was co-treated with gonadotrophins (Oliveira et al., 2001; Husein et al., 2007). Equine chorionic gonadotrophin (eCG) and follicle stimulating hormone (FSH) are the common gonadotrophins in oestrus synchronisation of domestic animals (Wildeus, 2000). However, eCG is more commonly used than FSH despite previous reports of the development of antibodies against eCG which adversely affects subsequent fertility (Baril et al., 1996). On the other hand, there are no reports of similar effects associated with the use of FSH (Viudes De Castro et al., 2009). Thus, there is a need to evaluate the use of FSH in oestrus synchronisation in goats.

Most research on oestrus synchronisation had been conducted in the developed temperate countries such as France and USA, in seasonally polyoestrous goat breeds and in temperate climates (Baril et al., 1993; Wildeus, 2000; Whitley and Jackson, 2004). However, it is necessary to observe the oestrus response in oestrus synchronised goats under the conditions they are raised. Consequently, many previous oestrus synchronisation studies in goats have been carried out in many countries under different management and environmental conditions (Ahmed et al., 1998; Amarantidis et al., 2004; De Santiago-Miramontes et al., 2009; Zhao et al., 2010).

Further studies could also be aided by serial ultrasonography which was introduced as a safe tool to monitor the growth and regression of ovarian antral follicles, repeatedly in the same animal regardless of their depth within the ovary (Bartlewski et al., 2000a; Bartlewski et al., 2002; Filho et al., 2007). This has made ultrasonography the preferred technique for the study of follicular development in domestic animals. However, ultrasonographhy has not been used to monitor follicular development in naturally cycling or oestrus synchronised non-seasonal polyoestrous goats in the humid tropics. Thus, an accurate depiction of the relationship between follicle development and plasma reproductive hormones concentrations could provide valuable information which could be used as a tool to control reproductive cycles in tropical goat breeds.

Follicular dynamics is closely associated with ovarian factors, which include insulin-like growth factor-1 (IGF-1), their binding proteins, and proteases (Hwa et al., 1999; Monget and Bondy, 2000; Monget et al., 2002). The positive effects of IGF-1 on ovarian physiology and follicular development has been reported in sheep (Scaramuzzi et al., 1999). In cattle, close association was found between endocrine IGF-1 concentration and some reproductive traits such as early age at first calving and first service to conception (Yilmaz et al., 2006). These associations led to the suggestion that endocrine IGF-I levels could be used as a selection tool for reproductively high performing sheep and cattle (Roberts et al., 1990; Taylor et al., 2004). However, in this study, the focus was to determine the correlation between endocrine IGF-1 and ovarian follicular development during induced and natural oestrous cycle as a more reliable indicator of the relationship between them. Thus, a

better understanding of the effects of oestrus synchronisation on endocrine IGF-1 concentration would also aid in the assessment of the actual clinical significance and usefulness of the measurement of circulating IGF-1 concentration.

It was hypothesized that oestrus synchronisation with $PGF_{2\alpha}$ and P_4 increased antral follicular development and plasma IGF-1 concentration and gonadotrophins stimulate the follicular development, increased the percentage oestrus response and the ovulation rate in goats. Thus, the aim of this study was to examine the relationship between plasma IGF-1 concentrations and antral follicular development during the oestrous cycles of $PGF_{2\alpha}$ or P_4 synchronised and naturally cycling goats. The efficiency of oestrus synchronisation with prostaglandin $F_{2\alpha}$ and P_4 with and without gonadotrophins was also evaluated.

The specific objectives were:

1. To determine the effects of $PGF_{2\alpha}$ or P_4 on follicular dynamics in oestrus synchronised and naturally cycling goats.
2. To determine the relationship between follicular development and plasma IGF-1 concentration in oestrus synchronised and naturally cycling goats.
3. To determine the effects of eCG on preovulatory follicle growth and time of ovulation in non-seasonal, polyoestrous goats
4. To determine the effects of $PGF_{2\alpha}$ and P_4 with or without eCG and FSH treatments on oestrus response and ovulation rates in non-seasonal polyoestrous goats raised under hot and humid condition.

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