UNIVERSITI PUTRA MALAYSIA

FOLLICULAR WAVE DEVELOPMENT, OESTRUS INTERVAL AND OVULATION RATE IN TIMED-ARTIFICIAL INSEMINATED KEDAH-KELANTAN, BRAKMAS, CHAROKE AND CROSSBRED COWS

AZIZAH BT AMRI

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By

AZIZAH BT AMRI

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

January 2013

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DEDICATION

WITH APPRECIATION AND RESPECT,

THIS THESIS IS DEDICATED

TO

MY LOVELY HUBBY; AHMAD NAZRI BIN ABDUL RAHMAN, MY CHILDREN; NURSHAZWANI, AHMAD SYAZWAN, AHMAD SYAZMI, AHMAD SYAZLI, AHMAD SYAZNI, MY MOTHER; PUAN SAMNAH BT SULBI, MY SON IN LAW; MUHAMMAD FAHMI BIN ZULKIFLI, AND MY GRANDSON IMAN SHAHMI

WHO ALWAYS INSPIRED ME

AND

MADE IT

WORTHWHILE

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

FOLLICULAR WAVE DEVELOPMENT, OESTRUS INTERVAL AND OVULATION RATE IN TIMED-ARTIFICIAL INSEMINATED (TAI) KEDAH-KELANTAN, BRAKMAS, CHAROKE AND CROSSBRED COWS

By

AZIZAH BT AMRI

January 2013

Chairman: Professor Abdul Wahid bin Haron, PhD

Faculty: Veterinary Medicine

The demands for beef, goat meat and mutton have been increased every year, and presently the national requirements for beef, goat meat and mutton are largely met by imports. Thus, steps should be taken to propagate the number of animals to reduce the loss in revenue through importation bills. The objectives of the study therefore were to (i) determine the differences in follicular wave development, (ii) determine the relationship and effect of ovarian follicular waves pattern on oestrus interval, ovulation time and pregnancy rate at observed oestrus, (iii) determine the oestrus interval and pregnancy rate at observed oestrus and (iv) develop the TAI and determine efficiency of frequency of TAI (single or dual) on the pregnancy rate of Kedah-Kelantan (KK), Brakmas (BK), Charoke (CK) and crossbred cattle following controlled internal drug release (CIDR) and prostaglandin (PGF) treatments. The hypothesis of the study is TAI at right ovulation will result in higher conception and/or pregnancy rates then AI of observed oestrus (a.m.-p.m. rules). All experiments used a single synchronisation protocol where cows were

inserted with CIDR® containing 1.38 g progesterone (P_4) for 7 days and given a intramuscular injection a synthetic PGF analogue of 25 mg PGF (Estrumate®, Schering – Plough Animal Health, Australia) two days prior to CIDR removal. Both ovaries of thirty cows comprised of Kedah-Kelantan (KK) and its crosses, Brakmas (BK) and Charoke (CK) were examined with a portable ultrasound machine at 6 hours interval in one duration of oestrous cycle to determine follicular development (FD). A total of 35 KK and its crosses were used to determine the interval between removal of CIDR® to oestrus and ovulation, AI and pregnancy diagnosis (PD) at the observed oestrus. The oestrous behaviour was observed for 72 hours at two-hour intervals beginning 12 hours after CIDR® removal, and AI was carried out at 6 and 12 hours after oestrus was observed. In another study, 64 KK cattle and its crosses were used to estimate ovulation time, and 60 KK cattle and its crosses cows were used to determine the TAI based on the ovulation time obtained. The mean diameter of dominant ovulatory follicles after CIDR removal was 11.73 ± 0.07 mm, and newly developed follicles after ovulation emerged at 1.37 ± 0.16 days with a mean size of 5.1 ± 0.02 mm. The number of follicles at emergence was significantly higher in KK (4.4±0.63, P=0.036) compared to BK (3.2±0.36) and CK (2.6±0.37). The mean interval from removal of CIDR to oestrus and ovulation, and oestrous to ovulation were 52.97 ± 6.16 h, 94.42 ± 16.41 h and 53.75 ± 25.36 h, respectively. The pregnancy rate (PR) of observed oestrus was higher in CK (43.8%), followed by KK (31.3%) and BK (25%). The mean estimation time of ovulation of the four breed groups was 85.1±1.6 h (P=0.467) calculated from the removal of CIDR®. The proportion of animals pregnant was higher with AI at 56 h groups with percentage pregnancy of 20% followed by AI at 72 h (15%) and AI at 65 h (10%) groups. The single AI

has produced a pregnant of a proportion 25% compared 20% for the dual AI method. The results of the present study has found the follicular growth which followed a wave like pattern, and there were differences in the development of the first, second and third follicular wave (FW) in BK, CK and KK cattle. There was a tendency for second and third dominant follicles to emerge at the same time, and both competed with each other to develop. The DF's whose its granulose cells produce enough oestradiol concentrations would enhance the development of the luteinising hormone (LH) receptors for higher LH production to induce ovulation. The study provided additional knowledge on the oestrous synchronization of KK, BK, CK and crossbred cattle for TAI. In conclusion, due to the results of proportion of cows pregnant, the study has not agreed with the hypothesis that TAI at right ovulation will result in higher conception rates then AI of observed oestrus (a.m.-p.m. rules). However, it was suggested the oestrous synchronisation for TAI in present study rendered further refinement, adjustment and modification in order to obtain a higher result in a conception or pregnancy rate.

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

PEMBENTUKAN GELOMBANG FOLIKULAR, SELANG MASA OESTRUS DAN KADAR OVULASI DALAM PERMANIAN BERADAS SECARA TEPAT (TAI) DALAM LEMBU KEDAH-KELANTAN, BRAKMAS, CHAROKE DAN BAKA KACUKAN

Oleh

AZIZAH BT AMRI

Januari 2013

Pengerusi: **Professor Abdul Wahid bin Haron, PhD**

Fakulti: **Perubatan Veterinar**

Permintaan daging lembu dan kambing semakin meningkat setiap tahun, dan dewasa ini keperluan daging lembu dan kambing nasional diperolehi melalui pengimportan. Oleh sebab itu, langkah perlu diambil untuk menambah bilangan ternakan untuk mengurangkan kehilangan hasil melalui pengimportan. Objektif kajian ini adalah untuk (i) menentukan perbezaan gelombang dari segi pembentukkan gelombang follikular; (ii) menentukan hubungan dan kesan pembentukkan corak gelombang follikular ke atas selang estrus, masa ovulasi dan kadar kebuntingan berdasarkan pemerhatian estrus; (iii) menetukan selang antara CIDR, estrus, masa ovulasi dan kadar kebuntingan; dan (iv) membangunkan masa tepat permanian beradas (TAI) dan menentukan kecekapan kadar kebuntingan samada TAI perlu dilakukan sekali atau dua kali pada lembu baka Kedah-Kelantan (KK) dan kacukan KK yang dirawat dengan "*controlled internal drug release"* (CIDR) dan prostaglandin (PGF). Hipotesis kajian adalah TAI yang dibuat pada ovulasi yang betul memberikan keputusan kadar konsepsi dan/atau

kebuntingan yang tinggi berbanding AI yang dibuat berdasarkan pemerhatian estrus (peraturan a.m.-p.m.). Kesemua kajian di dalam bab ini menggunakan protokol penyergaman estrus yang sama di mana lembu dirawat dengan memasukkan CIDR yang mengandungi 1.38 g progesterone (P_4) selama 7 hari dan analog PGF sintetik yang mengandungi 25 mg PGF (Estrumate®, Schering – Plough Animal Health, Australia) 2 hari sebelum CIDR dikeluarkan. Kedua-dua belah ovari 30 ekor lembu yang terdiri daripada Kedah-Kelantan (KK) dan baka kacukannya, Brakmas (BK) dan Charoke (CK) diperiksa dengan alat ultrabunyi mudah alih pada selang waktu 6 jam di dalam satu tempoh kitaran estrus sebelum menentukan pembangunan follikular. Sejumlah 35 KK dan baka kacukannya digunakan untuk menentukan waktu selang antara CIDR dicabut ke estrus dan ovulasi, AI dan diagnosa kebuntingan (PD) berdasarkan pemerhatian estrus. Tingkah laku estrus diperhatikan selama 72 jam (h) setiap selang waktu 2 h bermula 12 h selepas CIDR dicabut, dan AI dilaksanakan pada 6 dan 12 h selepas estrus diperhatikan. Dalam kajian yang lain, 64 ekor KK dan kacukannnya dibahagikan kepada empat kumpulan rawatan untuk menganggarkan masa ovulasi, dan 60 ekor KK dan kacukannya digunakan untuk menentukan TAI berdasarkan masa ovulasi yang diperolehi. Min diameter follikel dominan pengovulan selepas CIDR dikeluarkan adalah 11.73 ± 0.07 mm, dan folikel baru muncul pada 1.37 ± 0.16 hari dengan min saiz 5.1 ± 0.02 mm selepas ovulasi. Bilangan folikel yang muncul adalah sangat signifikant dalam KK (4.4±0.63, $P<0.05$) berbanding BK (3.2±0.36) dan CK (2.6±0.37). Min selang waktu antara CIDR di cabut dengan estrus dan ovulasi, dan estrus dengan ovulasi masing-masing adalah 52.97 ± 6.16 h, 94.42 ± 16.41 h dan 53.75 ± 25.36 h. Kadar kebuntingan berdasarkan pemerhatian estrus adalah tinggi di dalam CK (43.8%),

diikuti oleh KK (31.3%) dan BK (25%). Anggaran min masa ovulasi untuk empat jenis baka adalah 85.1±1.6 h (P=0.467) dikira bermula dari CIDR dicabut. Kadar kebuntingan adalah tinggi pada kumpulan AI di buat pada 56 h dengan peratus kebuntingan 20% diikuti oleh kumpulan AI dibuat 72 h (15%) dan AI yang dibuat 65 h (10%). Sementara AI dibuat sekali memperlihatkan kadar kebuntingan ternakan adalah 25% berbanding AI dibuat sebanyak dua kali iaitu 20%. Sebagai kesimpulan, keputusan kajian ini menunjukkkan pembangunan follikel adalah seperti pola gelombang, dan terdapatnya perbezaan di antara pembangunan gelombang pertama, kedua dan ketiga di dalam lembu BK, CK dan KK. Kajian ini memperlihatkan terdapatnya kecenderungan di mana follikel dominan kedua dan ketiga muncul pada masa yang sama dan kedua-duanya bersaing di antara satu dengan yang lain untuk membangun. Walaubagaimanapun, folikel dominan di mana sel granulosa berupaya menghasilkan kepekatan oestradiol yang cukup tinggi akan mempertingkatkan lagi pembangunan saraf menerima (*reseptor*) hormon penglutin (LH) untuk meningkatkan pengeluaran LH bagi mencetus ovulasi. Kajian menyediakan pengetahuan tambahan penyeragaman estrus ke atas lembu baka KK dan kacukan KK untuk melaksanakan TAI pada persekitaran Malaysia. Secara kesimpulannya, kajian ini tidak bersetuju dengan hipotesis yang dibuat dimana TAI pada ovulasi yang betul memberikan keputusan kadar konsepsi dan/atau kebuntingan yang tinggi berbanding AI yang dibuat berdasarkan pemerhatian estrus. Walaubagaimanapun, sinkronisasi estrus untuk TAI dalam kajian ini memerlukan pemurnian, penyelarasan dan perubahan yang lebih lanjut untuk mencapai keputusan kadar konsepsi dan/atau kebuntingan yang tinggi.

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I certify that a Thesis Examination committee has met on 8 January 2013 to conduct the final examination of Azizah binti Amri on her thesis entitled "Follicular Wave Development, Oestrus Interval and Ovulation Rate in Timed-Artificial Inseminated Kedah-Kelantan, Brakmas, Charoke and Crossbred Cows" 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 degree Doctor of Philosophy.

Members of the Examination Committee are as follows:

Rasedee @ Mat Abdullah, PhD
Professor
Faculty of Veterinary Medicine
Universiti Putra Malaysia
(Chairman)

Mohamed Ali Rajion, PhD
Professor
Faculty of Veterinary Medicine
Universiti Putra Malaysia
(Internal examiner)

Md Zuki Abu Bakar, PhD
Professor
Faculty of Veterinary Medicine
Universiti Putra Malaysia
(Internal examiner)

Petrus Leonardus Agnes Maria, PhD
Senior Lecturer
Utrecht University
Holland
(External examiner)

NORITAH OMAR, PhD
Assoc. Professor and Deputy Dean
School of Graduate Studies
Universiti Putra Malaysia

Date: 26 June 2013

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

Abd Wahid Haron, PhD
Professor
Faculty of Veterinary Medicine
Universiti Putra Malaysia
(Chairman)

Mohamed Ariff Omar, PhD
Professor
Faculty of Veterinary Medicine
Universiti Putra Malaysia
(Member)

Halimatun Yaakub, PhD
Associate Professor
Faculty of Agriculture
Universiti Putra Malaysia
(Member)

Sabrina Sukardi, PhD
Associate Professor
Faculty of Medicine and Health Science
Universiti Putra Malaysia
(Member)

BUJANG BIN KIM HUAT, PhD
Professor and Dean
School of Graduate Studies
Universiti Putra Malaysia

Date:

DECLARATION

I hereby 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 of concurrently, submitted for any other degree at Universiti Putra Malaysia or other institutions.

AZIZAH BT AMRI

Date: 8 January 2013

TABLE OF CONTENTS

LIST OF TABLES

LIST OF FIGURES

LIST OF PLATES

LIST OF ABBREVIATIONS

The following abbreviations are used in the thesis with or without definition.

AI	Artificial insemination
ANOVA	Analysis of variance
ARC	American Research Council
B/Bo	Percent bound
BST	Bovine somatotrophin
BK	Brakmas
BW	Body weight
cAMP	Cyclic AMP
CL	Corpus luteum
CF	Crude fibre
CP	Crude protein
CPM	Count Per minute
CIDR	Controlled internal drug release
CK	Charoke
D	Days
DFs	Dominant follicles
DM	Dry matter
EE	Ether extract
E/P	Estradiol prostaglandin
F	Fasting
FSH	Follicle Stimulating Hormone
FW	Follicular wave

GE	Gross energy
GSM	Single maintenance group
GDM	Double maintenance group
GnRH	Gonadotropin-releasing hormone injection
h	Hour
IBHK	National Institute of Biotechnology
IGF	Insulin-like growth factors
IQC	International Quality Control
KK	Kedah-Kelantan
Kg	Kilogram
LH	Luteinizing Hormone
M	Maintenance
MARDI	Malaysia Agiculture Reseach and Deelpment Institute
MGA	Melengesterol acetate
MJME	Mega Joule Metalbolisme energy
MJ	Mega Joule
mL	Milliliter
mL	Microliter
mg	Microgram
MHz	Mega hertz
NEM	Net energy for maintenance
ng/mL	Nanogram per milliter
NRC	National Research Council
ODB	Oestradiol benzoate
OPF	Oil Palm Fronds

$PGF_{2\alpha}$	Prostaglandin $F_{2\alpha}$
P_4	Progesterone
PKC	Palm Kennel Cake
POME	Palm mill Effluent
PD	Pregnancy diagnosis
SBM	Soyabean meal
RIA	Radioimmunoassays
^{125}I	Deionized iodin 125
TAI	Timed Artificial Insemination
TC	Total count
SPSS	Statistical analysis for Social Science
IVP4	Intravaginal progesterone releasing inserts
W	Weight

LIST TIMED ARTIFICIAL INSEMINATION PROTOCOLS

ABBREVIATIONS

PGF	Prostaglandin ($PGF_{2\alpha;}$ Lutalyse®, Estrumate®, ProstaMate®)
GnRH-PG (Select Synch)	Gonadotropin-releasing hormone injection (GnRH;Cystorelin®, Factrel®, Fertagyl®, OvaCyst®) followed in 7 days with an injection of $PGF_{2\alpha}$.
PG 6-day	$PGF_{2\alpha}$ followed in 3 days with an injection of GnRH at CIDR insertion;6 days later CIDRs are removed coincident with the administration of $PGF_{2\alpha}$
Ovsynch	Two injections of gonadotropin releasing hormone (GnRH) are given 9 days apart, with a $PGF_{2\alpha}$ injection given 7 days after the first GnRH treatment
Cosynch	Modification of Ovsynch with addition of second injection of GnRH and AI are performed at the same time
7-day CO-Synch + CIDR	GnRH is administered at CIDR insertion on day 0, followed 7 days later with CIDR removal, and $PGF_{2\alpha}$. Insemination is performed 66 hours after CIDR® removal and $PGF_{2\alpha,}$ with GnRH administered at AI.
5-day CO-Synch + CIDR	GnRH is administered at CIDR insertion on day 0, followed 5 days later with CIDR removal, and $PGF_{2\alpha}$. A second injection of $PGF_{2\alpha}$ is administered 8 ± 2 hours after CIDR removal and the first injection $PGF_{2\alpha}$. Insemination is performed 72 hours after CIDR removal and the first injection $PGF_{2\alpha,}$ with GnRH administered at AI.

CHAPTER 1

INTRODUCTION

Ruminant production has recorded poor growth rates and presently the national requirements for beef, goat meat and mutton are largely met by imports. The Department of Veterinary Services statistical analysis reported the consumption of beef has increased from 120, 270 metric tonnes in 2001 to over 168, 273 metric tonnes in 2011 with the increment of per capita consumption of 5.1 in 2001 to 5.71 in 2011. With the increment of the demand, steps should be taken to propagate the number of animals to reduce the loss in revenue through importation bills.

The information of when the cow showed the onset of oestrus renders the possibility to estimate the optimum time to conduct artificial insemination (AI). In addition, it is also important to detect the duration of oestrus in cattle in order to reduce the cost when practicing the AI technique (Cobert *et al.*, 1999). Therefore, the observation of oestrous signs or behaviour is beneficial in order to conduct the AI at the correct time to increase pregnancy rates (Colazo *et al.*, 2004a, b).

Synchronisation of oestrus in cattle can facilitate the use of AI by reducing the time needed for the detection of oestrus compared to cattle entering oestrus spontaneously. It is also an effective means of increasing the proportion of females that become pregnant early with shorter calving seasons and produce uniform calf crops (Dziuk

and Bellows, 1983). It was reported that up to 85% of cattle enter oestrus between 36 and 60 hour after being synchronised with progestin release subcutaneous implants or intravaginal progesterone releasing inserts (IVP_4) (Diskin *et al*., 2002). Others have reported over 90% of cattle can be induced to come into oestrus within 24 hours (Cavalieri *et al.*, 2003; Cavalieri *et al.*, 2004)

The ability of animals to conceive also depends on the right time to introduce the semen into the vagina of the female. Therefore, the oestrous detection, time and technique of AI, and the quality of semen are very important. It is known that insemination between 12-24 hours after the beginning of standing oestrous is the best time to ensure that sperm arrive at the site of fertilization before ovulation (Peters and Ball, 1995a). Therefore, with the information gathered, researchers are able to establish the optimum time to conduct AI.

Recently, research in cattle reproduction has focused on the development and growth of an ovulatory follicle within the ovary. In order to improve the efficiency of breeding techniques such as AI and embryo transfer, the knowledge regarding the development and control of the ovarian follicular development is required. Although there are few studies on ovarian follicular development in *Bos indicus* cows (Rhodes *et al.* 1995a) and none of them involved the Malaysian local breeds such as Kedah-Kelantan, Brakmas and Charoke**.**

Breed improvement through the increased rate of gamete propagation and dissemination are well known in the use of AI in livestock industry. The AI has been applying in the ruminant industry with varying degree of application. Introduction of AI in the ruminant industry had been conducted in the last three decades. It is used when bulls are not available and the technique beneficial in utilization of genetically superior males. Unfortunately, the use of AI in ruminants resulted in poor pregnancy rates. It was estimated to be about 20 to 30% successful.

During the oestrous cycle, a number of follicles are selected to complete differentiation, luteinisation and ovulation. Follicular development involves three stages, which are recruitment, selection and dominance. The process whereby a cohort of follicles is formed from a larger number of smaller follicles is termed recruitment. The single follicles in each cohort will be selected and continue to grow to become dominant and suppress the growth of other follicles in the cohort (Rajakoski, 1960; Adams, 1994; Adams and Pierson, 1995; Pierson and Ginther, 1987; 1988b).

The study of follicular dynamics can be carried out by visualizing bovine ovaries using transrectal ultrasonography (Pieterse *et al*., 1991; Ginther, 1987, Fortune *et al*., 1988b; Pierson and Adams, 1988b; 1999; Vassena *et al*., 2003; Vinoles *et al*; 2004). It is performed in order to determine large variations in the turnover of follicles and timing of ovulation. Transrectal ultrasonic imaging of ovarian follicles reveals that most oestrous cycles in cows have two, three or four follicular waves (FW). Follicular growth is a process where a cohort of follicles began to mature and grow under the

influence of sufficient pituitary gonadotropic stimulation to permit progression towards ovulation (Ireland *et al*., 1983a; 1983b; 1987; Lucy *et al*., 1992; Machatkova *et al*., 2000). The FW are first detected on 4-5 mm follicles and can be recognized in a series of follicular development at the growing, static and regressing phases. The period from emergence of a wave to apparent cessation of progressive growth is defined as the growth phase. Plateau phase is the period from cessation of growth to the apparent onset of progressive decreases in diameter and regressive phase can be defined as the period after the end of the plateau phase (Rajakoski, 1960). The researcher will then benefit from the development of a controlled breeding program, which combines synchronisation of ovulation with the ability to conduct a timed AI (Peters and Ball, 1995; 1997a; b; 1998; Fricke *et al*., 1998; Gabriel *et al*., 2006) without the need for oestrus detection.

Hence, studies on oestrous synchronisation that incorporated timed-AI have advanced in the oestrous cycle and induce ovulation at a pre-determined time should be considered in order to improve the success of the AI technique. Pregnancy rates matched or exceeded pregnancy rates to AI and twice daily heat detection allowed the opportunity to incorporate these technologies without too much time and labour. In addition, the technique allowed all animals to be managed in groups rather than individually. The need to improve the timing of AI which will result in high conception rate on a predictable basis is important. Hence, programs that facilitate the mass breeding of all animals at a predetermined time (timed-AI; TAI) are needed in order to increase the efficiency of the animal industry. In this study, we will monitor the follicular development and estimate the ovulation time in local breeds of beef

cattle. The hypothesis of the study is that timed-AI at right ovulation will result in higher conception and/or pregnancy rates then AI of observed oestrus (a.m.-p.m. rules) in beef cattle. The need to improve timing of AI which will result in high conception and/or pregnancy rate on a predictable basis is important. Therefore objectives of this study are to:

(i) determine the differences in follicular wave development in Kedah-Kelantan, Brakmas and Charoke cattle

(ii) determine the relationship and effect of ovarian follicular waves pattern on oestrus interval, ovulation time and pregnancy rate of observed oestrus in Kedah-Kelantan and crossbred cows

(iii) determine the CIDR, oestrus and ovulation interval, and pregnancy rate of observed oestrus in Kedah-Kelantan and crossbred cows

(iv) develop the TAI and determine efficiency of frequency of TAI (single or dual) on the pregnancy rate in KK and KK crossbred of cattle.

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