UNIVERSITI PUTRA MALAYSIA

OESTRUS BEHAVIOUR, FOLLICULAR GROWTH AND OVULATION TIME IN COWS BIOSTIMULATED BY BULLS

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FPV 2011 20

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By

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Thesis Submitted to the School of Graduate Studies, Universiti Putra Malaysia, in Fulfilment of the Requirements for the Degree of Master of Veterinary Science

November 2011

DEDICATION

Dedicated to my father Nguyen Kim Son and my mother Huynh Thi Chuc

Abstract of thesis presented to the Senate of Universiti Putra Malaysia in fulfilment of the requirement for the degree of Master of Veterinary Science

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Chairman: Assoc. Prof. Rosnina Yusoff, PhD

Faculty: Veterinary Medicine

Pheromonal communication plays an important role in animal behaviour and reproductive processes; biostimulation is a kind of pheromonal transmitting information, coined to express the stimulatory effect of a male on a female's oestrus and ovulation. The biostimulation technique offers a potentially useful and practical way to improve reproductive efficiency in cattle breeding. Therefore, the study was carried out to depict the effects of biostimulation by exposing cows to bulls on oestrus behaviour, follicular growth, and ovulation time. The experiment was conducted on 42 KK cows biostimulated by 3 KK bulls. The cows were divided into three groups: primiparous (PB) and multiparous cows (MB) with bulls and multiparous cows without bulls (CWB). Before start of the research, pregnancy status of the cows was checked through ultrasonography and then, the selected non-pregnant cows were synchronized with CIDR. The cows in each group were housed

in three separate paddocks, approximately 1 kilometre away from each other. On the third day of CIDR treatment, 3 bulls were firstly mixed with the cows in the MB group and then, secondly in the PB group. In the CWB group, AI was performed 12 hours after cows had displayed standing heat. Signs of oestrus were observed and scored based on the scoring system developed by Van Eerdenburg *et al.* (2002). After CIDR removal, follicle growth and ovulation time were determined by scanning the ovaries twice a day for 4 consecutive days. Blood samples were collected 16 times during 45 days for determining plasma progesterone concentration. Pregnancy status of the cows was also checked by ultrasonography on the 35th day from CIDR removal. The overall means percentage of cows in oestrus of the three groups was 90.52% with no significant difference among the groups. However, PB and MB groups scored higher ($P<0.05$) in signs of oestrus points for intensity of oestrus than CWB. Three main signs commonly displayed by majority of the cows were mounting another cow, being mounted but not standing and standing heat. Cows in the PB and MB groups displayed being mounted but not standing and mounting another cow significantly ($P<0.05$) more intense than CWB group. However, standing heat exhibited by oestrus cows was not significantly different among the three groups. PB group (23.64±4.15 hours) had significantly longer ($P<0.05$) duration of oestrus when compared with MB (12.69±2.36 hours) and CWB (13.40±1.50 hours) groups, respectively. In this study, the proportion of ovulation was quite high (90% to 95%) but not significantly different between groups. Ovulation occurred in PB cows significantly longer ($P<0.05$) than CWB cows from onset of being mounted but not standing and onset of mounting. However, there was no difference in ovulation time from standing heat between groups. No significant difference was also observed in growth of follicle and average daily growth rate in

each group. However, dominant follicle size was smaller in PB cows than MB cows. Progesterone concentrations were very low (around 0.7 ng/ml) on day 10 until day 12 in all groups. In PB cows, progesterone concentrations still remained at low levels until day 15. However, it increased slowly in MB and CWB cows. Conception rate in PB and MB groups was significantly higher than CWB group (66.67% and 69.23% vs 23.53%, $P<0.05$). In conclusion, biostimulation prolonged the duration of oestrus, increased the intensity of oestrus, a number of cows expressed being mounted but not standing and the frequency of cows displayed mounting behaviour in primiparous cows. However, duration of oestrus, intensity of oestrus, growth of follicle, dominant follicle size and ovulation time were not influenced by biostimulation in multiparous cows. Higher conception rate occurred in natural mating than AI.

Abstrak tesis yang dikemukakan kepada Senat Universiti Putra Malaysia sebagai memenuhi keperluan untuk ijazah Master Sains Veterinar

PERILAKU OESTRUS, PERTUMBUHAN FOLIKEL DAN MASA OVULASI BAGI BETINA OESTRUS YANG DIBIORANGSANG OLEH LEMBU JANTAN

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Komunikasi feromon memainkan peranan yang penting dalam tingkah laku dan proses pembiakan haiwan; bioperangsangan adalah sejenis penghantaran maklumat feromon, yang dicipta untuk menunjukkan kesan perangsangan pada jantan terhadap estrus dan pengovulanan betina. Kaedah bioperangsangan adalah kaedah yang berpotensi dan praktikal dalam meningkatkan kecekapan pembiakan di ladang ternakan. Oleh itu, penyelidikan ini telah dijalankan untuk menentukan kesan bioperangsangan terhadap lembu betina terhadap perilaku estrus, pertumbuhan folikel, dan masa pengovulanan di kalangan lembu pedaging Kedah-Kelantan. Kajian dijalankan terhadap 42 ekor lembu betina dan 3 ekor lembu jantan; lembu betina dibahagikan kepada tiga kumpulan: primipara bersama jantan (PB), multipara bersama jantan (MB) dan lembu multipara tanpa penjantan (CWB). Pertamanya, haiwan diperiksa bagi menentukan status kebuntingan menggunakan pengimbas ultrabunyi dan diselaraskan estrus dengan CIDR. Ketiga tiga kumpulan lembu ini

ditempatkan di petak rumput, jarak sekitar 1 km jauh antara satu sama lain. Pada hari ketiga rawatan CIDR, 3 ekor lembu jantan telah dicampurkan dengan lembu betina dari kumpulan PB dan MB sebagai bioperangsangan. Lembu betina CWB, pula diinseminasi 12 jam selepas lembu menunjukkan estrus berdiri. Petanda estrus dicerap dan dicatatkan berasaskan sistem penilaian yang dihasilkan oleh Van Eerdenburg dan rakan kerja (2002). Selepas CIDR dicabut keluar, perkembangan folikel dan masa ovulasi ditentukan dengan pengimbasan ultrabunyi terhadap ovari lembu, 2 kali sehari selama 4 hari berturut-turut. Kadar konsepsi dikesan pada hari ke-35 selapas CIDR dicabut keluar. Secara puratanya, peratusan lembu estrus untuk ketiga-tiga kumpulan adalah 90.52%. Walau bagaimanapun, tidak ada perbezaan berkepentingan di kalangan lembu dalam setiap kumpulan. Lembu dari kumpulan PB dan MB memperolehi skor tinggi lagi keamatan estrus berbanding dengan CWB. Secara individu, kebanyakan lembu pada semua waktu menunjukkan tiga ciri utama estrus: memanjat lembu lain, dipanjat dan estrus berdiri. Kumpulan PB dan MB menunjukkan tanda kelakuan estrus yang dipanjat tetapi tidak berdiri dan memanjat lembu lain adalah lebih ketara berbanding dengan CWB ($P<0.05$). Namun, estrus berdiri yang ditunjukkan oleh lembu yang estrus adalah tidak jauh berbeza antara kumpulan. Kumpulan PB (23.64±4.15 jam) menunjukkan tempoh ghairah yang lebih panjang ($P<0.05$) dibandingkan dengan MB (12.69±2.36 jam) dan CWB (13.40±1.50 jam). Dalam kajian ini, kadar ovulasi adalah tinggi (90% hingga 95%) tetapi tidak ada perbezaan yang ketara antara setiap kumpulan. Ovulasi berlaku lebih lama pada lembu PB daripada CWB secara ketara dalam semua peristiwa ($P<0.05$). Walau bagaimanapun, tidak ada perbezaan yang ketara dalam masa ovulasi sejak ditingkatkan antara PB dan MB, dari aktiviti memanjat antara MB dan CWB, dari estrus berdiri antara PB dan MB atau antara MB dan CWB. Tidak ada perbezaan

yang ketara telah diperhatikan untuk pertumbuhan folikel dan purata perkembangan harian untuk setiap kumpulan. Walau bagaimanapun, saiz dominan untuk folikel adalah lebih kecil pada PB berbanding dengan MB. Kepekatan progesteron adalah sangat rendah (dalam lingkungan 0.7 ng/ml) pada hari ke-10 sehingga hari ke-12 dalam semua kumpulan. Dalam lembu PB, kepekatan progesteron masih kekal pada tahap yang rendah sehingga hari ke-15. Akan tetapi, ia meningkat secara perlahan-lahan pada lembu MB dan CWB. Kadar konsepsi di PB dan MB adalah lebih tinggi daripada CWB dengan ketara (66.67% dan 69.23% vs 23.53%, $P<0.05$). Kesimpulanya, bioperangsangan memanjangkan tempoh bagi oestrus, meningkatkan intensiti bagi oestrus, segolongan lembu dinyatakan telah dicagakan tetapi tidak ditindikan dan kadar untuk lembu menunjukkan tingkah laku bercagak di primipara lembu. Akan tetapi, tempoh oestrus, keamatan bagi oestrus, perkembangan folikel, saiz dominan folikel dan masa ovulasi tidak dipengaruhi oleh kesan bioperangsangan di lembu multipara. Kadar konsep yang lebih tinggi berlaku di pengawan semulajadi berbanding dengan AI.

ACKNOWLEDGEMENTS

I would like to thank my committee chairperson Assoc. Prof. Dr. Rosnina Yusoff who provided me the opportunity and valuable support to pursue my master degree at Universiti Putra Malaysia and for her guidance during my academic program. I am deeply indebted to my committee members Prof. Dr. Abdul Aziz Saharee, Prof. Dr. Mohamed Ariff Bin Omar, and Assoc. Prof. Dr. Gurmeet Kaur Dhaliwal for the guidance, the constructive criticism, meaningful advices and for the scientific knowledge they have shared during my research progress.

I also would like to thank the staff at the School of Graduate Studies and at the Faculty of Veterinary Medicine, Universiti Putra Malaysia for providing good services. My appreciation also goes to Mr. Yap Keng Chee, Mr. Mohd Fahmi bin Mashuri for their assistances, and my lab mates, Dr. Mohamed Bukar, Dr. Yimer Nurshusein, Dr. Abdul Malik and Dr. Khumran Armiya'u Mada and all my other friends at the faculty.

I wish to thank the staff at the Government farm in Tanah Merah, Kelantan who have assisted me in the field work and to my housemates, Mr. Beh Boon Kee, Mr. Yeap Swee Keong for helping me to write the abstract in Bahasa Malaysia.

I am also in debt to Assoc. Prof. Dr. Luu Huu Manh, Dr. Nguyen Huu Hung, Dr. Huynh Kim Dieu, Dr. Do Trung Gia, Dr. Nguyen Duong Bao, lecturers at the Faculty of Veterinary Medicine, CanTho University; Mr. Huynh Ky, Mr. Le Vinh Thuc, Mr. Tran Than, Mr. Nguyen Phuoc Dang and other Vietnamese friends for the support and encouragement during my study in Malaysia.

Finally, my deepest appreciation goes to my parents, and my relatives for the enormous moral support they have given me.

This thesis was submitted to the Senate of Universiti Putra Malaysia has been accepted as fulfillment of the requirement for the degree of **Master of Veterinary Science**. 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 not concurrently, submitted for any other degree at Universiti Putra Malaysia and at any other institutions.

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NGUYEN PHUC KHANH

Date: 8 November 2011

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LIST OF ABBREVIATIONS

AI	Artificial insemination
ANOVA	Analyses of variance
BCS	Body condition score
CIDR	Controlled internal drug releasing
CL	Corpus luteum
CWB	Cow without bulls
ELISA	Enzyme-linked Immunosorbent Assay
FAO	Food and Agriculture Organization
FSH	Follicle Stimulating Hormone
FTAI	Fixed time artificial insemination
IGFs	Insulin-like growth factors
GnRH	Gonadotropin-releasing Hormone
LH	Luteinizing hormone
LID	Local Indian Dairy
MB	Multiparous cow with bulls
MHz	Megahertz
P_4	Progesterone
$PGF_{2\alpha}$	Prostaglandin $F_{2\alpha}$
MOET	Multiple ovulation with embryo transfer
NSB	Non-specific binding
PB	Primiparous cow with bulls
PPL	Palpation per rectum
PRID	Progesterone releasing intravaginal device
RIA	Radioimmunoassay

SEM	Standard error of the mean
SPSS	Statistical package for the social sciences

CHAPTER 1

INTRODUCTION

According to FAO, the cattle population in Malaysia was estimated at 790,000 head in 2008 (FAO, 2008). Apart from the indigenous breed, the Kedah-Kelantan (KK), the other popular breeds that are raised include the Friesian-Sahiwal cross (Mafriwal), the Local Indian Dairy (LID), the Droughtmaster, and the Brahman. Among these breeds, the KK cattle constitute 80% of the cattle population. The name KK is derived from the first alphabet of the names of two north-eastern states of Kedah and Kelantan. They are bred widely in the north and eastern regions of peninsular Malaysia and in southern Thailand. The KK cattle are small and compact with a reddish dun or grey and black haircoat. The main purpose of rearing the KK cattle is for meat production (Devendra, 1973).

Successful breeding of cattle contributes to the economic boost of a country. Application of breeding techniques such as artificial insemination (AI), oestrus synchronization and induction of multiple ovulations followed by embryo transfer (MOET) have been widely used in many countries (Bearden *et al.*, 2004). This helps farmers to profit from increased milk and meat production. Flores *et al.* (2006) reported that Brahman cows synchronized with CIDR (controlled internal drug releasing) combined with the administration of $PGF_{2\alpha}$ on the day of CIDR removal had increased the number of mounts received, improved number of cows in oestrus, enhanced conception rates and normalized interoestrus intervals in anoestrus cows. However, despite the progress in reproductive technologies, oestrus detection still

remains a critical step for successful implementation of AI, oestrus synchronization, and the MOET technique.

For successful fertilization, determination of time from insemination to ovulation is indispensable. When insemination of a cow takes place too early or too late in relation to ovulation, the conception is adversely affected. Therefore, to determine the time of insemination or ovulation, observation of behavioral oestrus is pertinent. Signs of oestrus include standing to be mounted, sniffing the vagina of another cow, and resting of chin and mounting behaviour (Roelofs *et al.*, 2005). Detection of oestrus behavioral signs must be done accurately and efficiently and these depend on certain factors such as frequency as well as duration of oestrus and timing of observable oestrus periods. It is noteworthy that factors, such as management practices, housing environment, temperature, nutrition, genetic factors, age and physiological status, can affect the manifestation of oestrus behavioral signs (Galina and Orihuela, 2007).

In addition to observation of oestrus behavioral signs, ultrasound is another method that has been successfully applied to determine the correlation between signs of oestrus and ovulation time. As is generally known, not all cows show oestrus behavioral signs; cows with silent oestrus could be checked to determine ovulation time by ultrasonography. This will contribute to the success of determining the optimal time for artificial insemination. Hanzen and colleagues (2000) reported that follicles of less than 5 mm can be identified by ultrasonography. In addition, ultrasonography can be used to detect early pregnancy more correctly and effectively when compared with palpation per rectum (PPR).

However, to purchase and own an ultrasound machine in third world countries like Laos, Myanmar and Vietnam in South East Asia and Ethiopia, Congo and Nigeria is a luxury. Moreover, the operator must be skilled and requires an enormous experience to interpret the images displayed on the monitor. The use of ultrasonography in a big herd can be time-consuming and stressful to the operator and the animals. Thus, for now, to detect oestrus, a quick, easy and cheaper method is essential in countries like Myanmar, Laos, Cambodia and Vietnam.

Biostimulation of cows is a stimulatory technique that uses a stud bull to enhance oestrus expression and ovulation through genital or pheromonal stimulation. This improves the detection of oestrus behavioral signs. Pheromones are air-borne chemical substances excreted via urine and faeces of animals or by subcutaneous glands resulting in a specific behaviour or physiological change in the recipients endocrine or reproductive system (Doty, 1976; Izard, 1983). Close physical contact with bulls can cause anoestrus cows and postpartum lactating cows to resume postpartum ovarian cycling activity (Custer *et al.*, 1990; Rekwot *et al.*, 2000; Berardinelli and Joshi, 2005a). By exposing beef cows to bulls, the postpartum anovulatory interval can be reduced (Berardinelli and Joshi, 2005a). Berardinelli *et al.* (2005b) showed that pheromones, present in bulls' excretory products, were the mediated factors which hastened postpartum resumption of luteal function.

Roelfs *et al.* (2005) reported that there was a correlation between oestrus behavioral signs and time of ovulation in cattle. Another research showed that there was no variation in the growth of follicles, diameter of ovulatory follicles and average growth rate (Maquivar *et al.*, 2007). However, these studies were conducted without

the presence of bulls. The question here is whether the presence of bulls can stimulate oestrus in those cows for improving chances of oestrus detection and also if the presence of bull affects the timing of ovulation.

Therefore, the present study was performed to appraise the biostimulation response of cows in terms of oestrus behavioral signs display, ovarian follicular dynamics and ovulation time in the primiparous and multiparous cows in the presence of bulls. The second objective was to compare the pregnancy rates between biostimulated and non-biostimulated cows.

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