UNIVERSITI PUTRA MALAYSIA

EFFECTS OF ENERGY LEVEL ON OESTROUS BEHAVIOUR AND FOLLICULAR DEVELOPMENT IN CATTLE IN MALAYSIA

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By

AZIZAH BT AMRI

Thesis submitted, to Graduate Studies, Universiti Putra Malaysia, in Fulfilment of the Requirement for the Degree of Master Science

February 2008

DEDICATION

WITH APPRECIATION AND RESPECT,

THIS THESIS IS DEDICATED

TO

MY HUSBAND AND CHILDREN: AHMAD NAZRI BIN ABDUL RAHMAN, NURSHAZWANI, AHMAD SYAZWAN, AHMAD SYAZMI, AHMAD SYAZLI, AHMAD SYAZNI, ALSO MY MOTHER PUAN SAMNAH BT SULBI

WHO INSPIRED ME

AND

MADE IT

WORTHWHILE

ABSTRACT

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

EFFECTS OF ENERGY LEVEL ON OESTROUS BEHAVIOUR AND FOLLICULAR DEVELOPMENT IN CATTLE IN MALAYSIA

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Chairman: Associate Professor Halimatun Yaakub, PhD

Faculty : Agriculture

The understanding of the development and growth of an ovulatory follicle within the ovary is important in order to improve the efficiency of breeding techniques such as artificial insemination (AI) and embryo transfer. The objectives of the study were to determine the effects of different levels of energy diets on characteristics of oestrous behaviour by visual observation, and follicular development in cows. The experiments were conducted to determine the oestrous behaviour by visual observation and the effects of dietary energy levels on follicular development in crossbred cows offered different levels and duration of energy diets. Thirty crossbred Kedah-Kelantan (KK) cows were divided into two groups, I) n=15; received grass with maintenance supplement and II) n=15; received grass with double maintenance supplement. Oestrous was initially synchronised using chloprostenol intra vaginal progesterone releasing device (CIDR-B®) containing 1.38 g progesterone for 7 days and administered 1 ml synthetic prostaglandin analogue 2 days before CIDR-B® removal. The cows were

observed for estrous behaviour continuously 24 hours daily for 72 hours immediately after CIDR-B® removal. The ovaries then were visualized using 7.5 MHz linear array transrectal transducer attached to a real-time, B mode portable ultrasound. Scanning was carried out from six hours at the beginning of estrous and repeated every six hours until ovulation. The day of first detection of a ≥ 4 mm follicle identified as a dominant follicle was taken as the first day of a follicular wave. During each ultrasound examination, a single blood sample was drawn and collected into 10 ml plain tubes for determination of progesterone concentration. A timed insemination was performed at 48 to 54 hrs after removal of CIDR-B® for all cows. Pregnancy diagnosis was performed using ultrasonography and rectal palpation techniques. In expressing oestrous behaviour characteristics, there were no differences between group of single maintenance (GSM) and group of double maintenance (GDM) ($p>0.05$) in the first cycle, while in the second cycle, characteristics of oestrous behaviour were clearly seen. The results obtained showed that approximately 33.3% of GSM and 40% GDM cows produced 2 waves of follicular development while 66.67% of GSM and 60% of GDM cows produce 3 waves of follicular development. Both GSM and GDM had equal number of cows producing 2 and 3 waves of follicular development. However, the number of waves of follicular development, length of estrous cycle, growth and regression rate of dominant follicle, and progesterone concentration between GSM and GDM were not significantly different ($p>0.05$). Therefore, the results from the experiment suggest that the higher and longer period of energy levels would not accelerate the estrous behaviour and increased the number of animals pregnant. From the study, it was concluded that cows offered double maintenance levels of energy diet was not significantly different from cows offered single maintenance levels of energy diet in terms of exhibit oestrous behaviour, follicular development and number of animal pregnant (P>0.05).

ABSTRAK

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

KESAN ARAS TENAGA KE ATAS PEMBENTUKAN FOLIKEL PADA LEMBU

Oleh

AZIZAH BT AMRI

Februari 2008

Pengerusi: **Profesor Madya Halimatun Yaakub, PhD**

Fakulti: **Pertanian**

Pemahaman mengenai pembentukan dan pembesaran folikel pengovul pada ovari adalah penting untuk memperbaiki kecekapan teknik pembiakan seperti permanian beradas (AI) dan pemindahan embrio. Objektif pembelajaran adalah untuk menentukan kesan aras tenaga diet yang berbeza ke atas perlakuan estrus melalui pemerhatian dan pembentukan folikel pada lembu betina. Eksperimen telah dijalankan untuk menentukan tingkahlaku estrus melalui pemerhatian secara visual dan kesan aras tenaga ke atas pembentukan folikel lembu betina baka silang. Tiga puluh ekor lembu betina Kedah-Kelantan (KK) baka silang dibahagikan kepada dua kumpulan, I) n=15, mendapat rumput dengan makanan tambahan penyelenggaraan dan II) n=15, mendapat rumput dengan makanan tambahan 2 kali ganda penyelenggaraan. Estrus diselaraskan dengan menggunakan CIDR-B® yang mengandungi 1.38 g progesterone selama 7 hari dan 1 ml analog prostaglandin sintetik diberi 2 hari sebelum CIDR-B® dikeluarkan dari vagina.

Perlakuan estrus dijalankan melalui pemerhatian 24 jam sehari secara berterusan selama 72 jam bermula dari CIDR-B® dikeluarkan. Kedua-dua ovari digambarkan menggunakan 7.5 MHz `linear array transrectal transducer' yang dilekapkan bersama ultrasound mudahalih `B-mode real-time'. Penemuan pertama folikel bersaiz ≥ 4 mm dikenalpasti sebagai folikel dominan dan diambil sebagai hari pertama gelombang bermula. Sampel darah dipungut dan disimpan ke dalam tiub kosong untuk penganalisaan kepekatan progesterone. Penetapan masa Inseminasi dijalankan pada 48 dan 54 jam setelah CIDR-B® dikeluarkan dari kesemua lembu betina. Diagnosa kebuntingan dijalankan dengan menggunakan kaedah ultrasnografi dan teknik palpasi rekta. Keputusan menunjukkan tidak ada perbezaan bererti diantara GSM dan GDM ($p>0.05$) pada ciri perlakuan estrus pusingan pertama, tetapi boleh dilihat secara jelas pada perlakuan estrus pusingan kedua. Keputusan yang diperolehi menunjukkan 33.3% dan 40% daripada lembu betina dari GSM dan GDM masing-masing sekurang-kurangnya menghasilkan 2 gelombang folikular manakala 66.7% dan 60% daripada GSM dan GDM masing-masing terdiri daripada 3 gelombang folikular. Kedua-dua GSM dan GDM mempunyai bilangan lembu betina yang sama terdiri dari 2 dan 3 gelombang pembentukan folikular. Walaubagaimanapun, bilangan gelombang pembentukan folikular, jangkamasa pusingan estrus, kadar pertumbuhan dan pengecutan folikel dominant, dan aras kepekatan progesterone di antara GSM dan GDM tidak ada mempunyai perbezaan yang bererti ($P>0.05$). Oleh yang demikian keputusan dari eksperimen mencadangkan kandungan yang tinggi dalam jangkamasa yang lama tidak mempercepatkan perlakuan estrus dan mempertingkatkan bilangan ternakan yang bunting (P>0.05). Dari pembelajaran ini, kesimpulan yang diperolehi adalah tiada hubungan yang bererti diperolehi di antara lembu betina yang diberi dua kali aras tenaga diet pengambilan makanan penyelenggaraan dengan lembu betina yang diberi sekali aras tenaga diet makanan penyelenggaraan berkaitan dengan perlakuan estrus, pembentukan folikel dan bilangan ternakan yang bunting (P>0.05).

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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
BW	Body weight
cAMP	Cyclic AMP
CL	Corpus luteum
CF	Crude fibre
CP	Crude protein
CPM	Count Per minute
CIDR/CIDR-B®	Chloprostenol intravaginal progesterone releasing device
d	Day
DFs	Dominant follicles
DM	Dry matter
EE	Ether extract
E/P	Estradiol prostaglandin
F	Fasting
FSH	Follicle Stimulating Hormone
GE	Gross energy
GSM	Single maintenance group
GDM	Double maintenance group

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
MINT	Institute for Nuclear Technology Research
MGA	Melengesterol acetate
MJME	Mega Joule Metalbolisme energy
MJ	Mega Joule
mL	Milliliter
μL	Microliter
μg	Microgram
NEM	Net energy for maintenance
ng/mL	Nanogram per milliter
NRC	National Research Council
ODB	Oestradiol benzoate
OPF	Oil Palm Fronds
PG	Prostaglandin analogue
P_4	Progesterone
PKC	Palm Kennel Cake
POME	Palm mill Effluent

W	Weight
MHz	Mega hertz
PD	Pregnancy diagnosis
SBM	Soyabean meal
RIA	Radioimmunoassays
^{125}I	Deionized iodin 125
TC	Total count
SPSS	Statistical analysis for Social Science
IVP4	Intravaginal progesterone releasing inserts
SAG	Sexually active group

CHAPTER 1

INTRODUCTION

Oestrus is the manifestation of sexual receptivity in the female mammal. Oestrous signs or behaviour is exhibited just before ovulation in cattle and is relatively short (2 to 3 % of the oestrous cycle) and usually last for 18-19 hours (ranging from 14 to 22 hours) (Hafez *et al.*, 2000). Each oestrous cycle in cattle is divided into four stages, pro-oestrus, oestrus, metoestrus and dioestrus.

Oestrus, the animal will show signs indicating the receptivity to mate. The female will attempt to mount, lick, show nervous signs and stand to be mounted by others. The vagina will become reddened with presence of mucous discharge (watery to thick) when it is close to ovulation (Roelofs *et al.,* 2005a). Various methods are being used to detect oestrous signs such as tail-paint and chalk, intravaginal and vulvar signs, electrical impedence, vasectomized bulls or continuous visual observation and radiotelemetry (Stevenson *et al.,* 1996; Xu *et al.,* 1998; Cavailieri *et al.,* 2003b). The information of when the cow showed the onset of oestrus renders possibility to estimate the optimum time to conduct artificial insemination. In addition, it is also important to detect the duration of oestrus in cattle in order to reduce the cost when practicing artificial insemination (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 detection of oestrus compared to cattle entering oestrus spontaneously. After progestin released subcutaneous implants or intravaginal progesterone releasing inserts (IVP4) were used to synchronize oestrus, up to 85% of cattle can be induced to enter oestrus between 36 and 60 hour after the end of treatment (Diskin *et al.*, 2002).

Nutrition has a key role in regulating reproductive processes in cattle. The effects of acute nutritional restriction in cattle are not known. Nutrition is an important element in altering the development diameter of follicles apart from its importance for development of dominant follicle size. It has been reported that the diameter size of dominant follicles decreased when feed is restricted to the animals (Murphy, 1991; Bergfeld, 1994; Rhodes, 1995). Apart from this Mackey *et al.* (1997a, 1999, 2000) concluded that an acute restriction in energy intake 3 to 6 days before ovulation decreased the growth rate and maximum size of the preovulatory dominant follicle. This subsequently resulted in fewer follicles emerging in the second follicular wave.

The main energy substrates used by ruminants are volatile fatty acids (acetate, butyrate and propionate) produced in the rumen. The concentration of volatile fatty acids profiles can be different due to differences in the rumen degradation of roughages (Archimede *et al.*, 1996). The highly degradable feed in the rumen, especially in excessive feeding with protein sources can reduce fertility in dairy cattle (Lanyasunya *et al.*, 2005). Butlers *et al.* (1996) indicated that high milk or plasma urea concentrations are clearly associated with decreased fertility in dairy cows. Alternatively, imbalances in the relative availability of protein and energy may affect efficiency of metabolism and energy status. In cattle, the relationship between dietary intake and progesterone (P_4) concentration in blood is controversial (Spitzer, 1986, 1978; McCann *et al.*, 1986). Lower P_4 could lead to prolonged follicular growth associated with lower fertility probably due to the decrease in oocyte quality (Mihm *et al.*, 1994, 2003).

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 *et al.*, 1995). Therefore, with the information gathered, researchers were able to establish the optimum time to conduct artificial insemination.

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.

During the oestrous cycle, a number of follicles are selected to complete differentiation 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 as recruitment. The single follicles in each cohort will be selected and continue to grow to become dominant and suppresses the growth of other follicles in the cohort (Rajakoski, 1960; Adams, 1994, 1995; Pierson and Ginther, 1987, 1988b).

The study of follicular dynamics can be obtained by visualizing bovine ovaries using transrectal ultrasonography (Pieterse *et al.*, 1991; 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 or three follicular waves. 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 follicular waves are first detected on 4-5 mm follicles and can be recognized in a series of follicular development as 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