



**UNIVERSITI PUTRA MALAYSIA**

***RESPONSE OF CONCENTRATE SUPPLEMENTATION ON OVULATION  
RATE AND PROGESTERONE RECEPTOR GENE IN GOATS***

**NUR HAFIZAH MOHAMMED**

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By

**NUR HAFIZAH MOHAMMED**

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

**May 2018**

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

## **RESPONSE OF CONCENTRATE SUPPLEMENTATION ON OVULATION RATE AND PROGESTERONE RECEPTOR GENE IN GOATS**

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**NUR HAFIZAH MOHAMMED**

**May 2018**

**Chairman : Mashitah Shikh Maidin, PhD**  
**Faculty : Science**

Nutritional supplementations on reproductive performance such as ovulation rate and embryo survival in sheep are well-documented, but there are still debates among researchers and farmers regarding this matter in goats. In goats, there were some evidences shown that high energy/protein supplementation does not promotes an increment on ovulation rate, but able to sustain the embryo survivability, thus maintain the pregnancy. Therefore, this study was conducted to examine the effect of short-term supplementation of concentrate (commercial concentrate; double maintenance) on size of ovary and corpus luteum (CL), ovulation rate in relation to live weight, body condition score (BCS) and changes of physiological parameter of the ovary and CL. Also, to determine the effects of protein supplement on mRNA expression of progesterone receptor (*PR*) gene, thus specific primer for *PR* gene was designed to qualify the presence of *PR* expression. In this study, 17 of female Boer goats were divided into two groups; 1) Control group received maintenance diet (commercial concentrate and Napier grass) and 2) Treatment Group received double maintenance diet (commercial concentrate-2M and Napier grass). The feeding treatment was started 5 days prior to CIDR removal (Day 0) for 25 days. On Day 19, ovaries were examined by ultrasound and ovulation rate were calculated by presence number of CL. The live weight and BCS were recorded every two weeks until animals were slaughtered (Day 27), the number and diameter of removed ovary and CL were measured. Results from this study demonstrated that short-term supplementation of concentrate had no effect on ovulation rate (Control =  $1.00 \pm 0.2$ ; Treated =  $1.25 \pm 0.2$ ;  $p > 0.05$ ) and no changes in live weight and BCS were observed throughout the experiment. In addition, in both Groups revealed that size of ovary does not correlated with size CL. We suggest that effect of concentrate supplementation inducing ovulation is relatively weaker in goats

compared to sheep. This might be due to low amount of energy level to enhance the ovulation rate. However, present results showed that the does treated with double maintenance of commercial concentrate able to influence the expression of *PR* gene in ovaries tissue. The expression of *PR* gene in supplemented goats was increased by 3.7 fold in CL and 2.3 fold in follicles tissue ( $p < 0.05$ ). Surprisingly, present result indicated that they expression of *PR* gene between left and right CLs was similar between groups. Hence, concentrate supplementation could promote growth and development of high quality predominant follicles and thus fully functional CL will be produced. We suggest that, concentrate supplementation is able to maintain the structural and functional integrity of CL tissue, thus production of progesterone concentration may not be affected. In this study, we concluded that concentrate supplementation are not able to increase ovulation rate of female goats but the supplement able to maintain the structural and function of CL tissue.

Keywords: Goats, concentrate, ovulation rate, gene expression, progesterone receptor

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

## REAKSI SUPLEMEN KONSENTRAT KEPADA KADAR OVULASI DAN GEN RESEPTOR PROGESTERON DI KAMBING

Oleh

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Pengaruh suplemen nutrisi terhadap prestasi pembiakan seperti kadar ovulasi dan kelangsungan hidup embrio di biri-biri telah banyak didokumentasikan dengan baik, tetapi masih terdapat perdebatan di antara para penyelidik dan petani berkenaan perkara ini mengenai kambing. Di kambing, terdapat beberapa bukti menunjukkan bahawa suplemen tenaga/protein yang tinggi tidak dapat menggalakkan peningkatan kadar ovulasi, tetapi mampu mengekalkan kelangsungan hidup embrio, justeru mengekalkan kehamilan. Oleh itu, kajian ini dijalankan untuk mengenalpasti kesan suplemen protein jangka pendek (kosentrat komersial; penyelenggaraan berganda) pada saiz ovari dan (corpus luteum) CL, kadar ovulasi berkaitan dengan berat hidup, skor keadaan badan (BCS) dan perubahan parameter fisiologi ovari dan corpus luteum (CL). Tambahan pula, untuk menentukan kesan suplemen protein pada *mRNA PR* gen, maka *primer* yang spesifik untuk gen *PR* direka untuk mengungkap kehadiran *mRNA PR*. Dalam kajian ini, 17 kambing betina baka Boer dibahagikan kepada dua kumpulan; 1) Kumpulan Kawalan menerima diet penyelenggaraan (kosentrat komersil dan rumput napier) dan 2) Kumpulan Rawatan menerima diet penyelenggaraan berganda (kosentrat komersil-2M dan rumput napier). Rawatan pemakanan bermula 5 hari sebelum CIDR dicabut (Hari 0) dan berlansung selama 25 hari. Pada Hari 19, ovari diperiksa menggunakan ultrasound dan kadar ovulasi dikira dengan mengira jumlah CL yang. Berat hidup dan BCS dicatat setiap dua minggu sehingga haiwan disembelih (Hari 31), bilangan dan diameter ovari yang dan CL yang dikeluarkan diukur. Keputusan dari kajian ini menunjukkan bahawa suplemen jangka pendek protein yang tinggi tidak mempunyai kesan ke atas kadar ovulasi (Kawalan =  $1.00 \pm 0.2$ ; Dirawat =  $1.25 \pm 0.2$ ;  $p > 0.05$ ) tanpa perubahan dalam berat hidup dan BCS sepanjang percubaan. Di samping itu, dalam kedua-dua kumpulan menunjukkan bahawa saiz ovari tidak berkaitan dengan saiz CL. Kami mencadangkan bahawa kawalan protein pemakanan tidak dapat meningkatkan kadar ovulasi di kambing kerana kesan daripada protein adalah lebih lemah

pada kambing berbanding biri-biri. Ini mungkin disebabkan jumlah protein yang diberikan adalah rendah dan tidak mencukupi untuk meningkatkan kadar ovulasi. Walau bagaimanapun, keputusan daripada kajian ini menunjukkan bahawa rawatan yang dilakukan dapat mempengaruhi ekspresi gen *PR* dalam tisu ovari. Ungkapan gen *PR* dalam kambing yang diberi 2 kali tambahan diet protein meningkat sebanyak 3.7 kali ganda di CL dan 2.3 kali ganda di tisu folikel ( $p < 0.05$ ). Tambahan pula, hasil dari kajian ini mendapati ekspresi gen *PR* antara CL kanan dan kiri adalah sama di antara kumpulan. Oleh itu, kami menyimpulkan bahawa suplemen pemakanan dapat menggalakkan pertumbuhan dan perkembangan folikel pra-dominan yang berkualiti tinggi dan menghasilkan CL yang mampu berfungsi dengan baik. Kami mencadangkan bahawa, suplemen berasaskan protein mampu mengekalkan integriti struktur dan fungsi tisu CL, dengan itu meningkatkan penghasilan hormon progesteron dan tidak menjejaskan kestabilan embrio dalam kambing. Ringkasnya, kami menyimpulkan bahawa suplemen berasaskan protein mungkin tidak memberi kesan kepada kadar ovulasi kambing betina tetapi mampu mengekalkan struktur dan fungsi CL.

Kata kunci: Kambing, suplemen kosentrat, kadar ovulasi, ungkapan gen, reseptor progesteron



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I certify that a Thesis Examination Committee has met on 18 May 2018 to conduct the final examination of Nur Hafizah binti Mohammed on her thesis entitled "Response of Concentrate Supplementation on Ovulation Rate and Progesterone Receptor Gene in 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 Master of Science.

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## LIST OF ABBREVIATIONS

%	Percentage
µg/µl	Microgram per microliter
µl	Microliter
°C	Degree Celcius
$2^{-\Delta\Delta Ct}$	Delta-delta-CT
ANOVA	Analysis of Variance
B-actin	Beta-actin
BCS	Body Condition Score
bp	Base pair
BW	Body weight
Cal	calories
cDNA	Complementary DNA
CIDR	Controlled Internal Drug Releasing Device
CL	Corpus luteum (singular) or Corpora lutea (plural)
CP	Crude protein
CT	Cycle threshold
DM	Dry matter
DNA	Deoxyribonucleic acid
F	Forward
FSH	Follicle stimulating hormone
g	Gram
GAPDH	Glyceraldehyde-3-phosphate dehydrogenase
GC	Guanine-cytosine
GnRH	Gonadotropin-releasing hormone
IACUC	Institutional Animal Care and Use Committee
IGF	Insulin-like growth factor
kcal/mol	Kilocalorie per mole
Kg	Kilogram
LH	Luteinizing hormone
MAFF	Ministry of Agriculture, Fisheries and Food
MANOVA	Multivariate analysis of variance

MARDI	Malaysia Agricultural Research and Development Institute
ME	Metabolized energy
ME <sub>m</sub>	Metabolizable energy for maintenance
mg	Milligram
MgCl	Magnesium chloride
min	Minute
MJ	Megajoule
mRNA	Messenger RNA
NCBI	National Center for Biotechnology Information
ng/μl	Nanogram per microlitre
ng/ml	Nanogram per millilitre
No.	Number
NRC	National Research Council
OD	Optical density
PCR	Polymerase Chain Reaction
PR	Progesterone receptor
RT-qPCR	Real-Time Quantitative Polymerase Chain Reaction
R	Reverse
RNA	Ribonucleic acid
Rpm	Revolutions per minute
rRNA	Ribosomal ribonucleic acid
sec	Second
SEM	Standard error of mean
SPSS	Statistical Package for the Social Sciences
T <sub>m</sub>	Melting temperature
U	Unit
UPM	Universiti Putra Malaysia
ΔG	Delta G

# CHAPTER 1

## INTRODUCTION

### 1.1 Introduction

Generally, to meet the increase in worldwide demand for goat products, more attention needs to be paid to their reproductive performance including ovulation rate and kidding rate. It is well-known that rate of offspring production is a one of the main contributor to economic success, particularly in Malaysia where the goat industry contribute heavily to the domestic economy. According to Department of Veterinary Services, Malaysia, the rate of self-sufficiency for goat production in Malaysia for year 2016 is only about 10.77%. This shows that Malaysia still heavily relies on imported goats. Literatures reported that nutritional control could enhance the livestock production and more refined feeding program known as “focus feeding” (Blache & Martin, 2009). Many studies on ‘focus feeding’ have been done in sheep and shows that introduction of short-term supplementation less than 10 days before mating to ewes promote the growth and development of domain follicles thus increase ovulation rate (Vinoles et al., 2010; Scaramuzzi et al., 2011; Somchit-Assavacheep et al., 2013; Dupont et al., 2014). With increment in ovulation rate, perhaps could increase the kidding rate.

Although in sheep the reproductive performances positively react towards nutritional control or supplementation, there are still debates among researchers and farmers regarding this matter in goats. There were some evidence that supplementations of high energy/protein does not increase ovulation rate in goats (Maidin et al., 2008; Haruna et al., 2009). A study done by Zarazaga et al. (2005) reported that although the secretion of pituitary hormones significantly different between high and low feeding groups, but the ovulation rate was not differs between groups in Payoya goats. In addition, although nutrition helps to promote folliculogenesis and increase ovulatory activity, but overfed animals prevent embryo to growth and develop; thus embryo mortality occurs during early pregnancy (Parr et al., 1993; Vinoles et al., 2012). In many studies, particularly in sheep reported that high supplementation before mating or during luteal phase decrease the progesterone concentration and give detrimental effect on the growth and development of embryo. However, Rhind et al. (1989) found no relationship between peripheral progesterone concentration and embryo survival. Recent study by Maidin et al. (2014) suggest that no progesterone clearance and embryo mortality occur on goats and concluded that in order to elucidate the effect of food intake on embryo survival it would be necessary to determine the effect of nutrition on the mediator of the hormone action.

Although progesterone hormones are known to act in unity with its receptor molecule to induce cellular effects, in major reproductive tissue involves in



modulating hypothalamus-pituitary-ovarian axis. However, none information exists regarding the influence of nutrition on expression of progesterone receptor (*PR*) in goats. In sheep, progesterone receptor amounts have been reported to be regulated in a cell type, in specific manner, during the estrous cycle and early pregnancy in sheep (Spencer & Bazer, 1995). The regulation of progesterone receptors under high plane nutrition also reported in endometrial of ewe (Sosa et al., 2004). This indicate that nutrition able to influence the progesterone action on the target cell by modify cellular responsiveness towards progesterone hormone. Therefore this study was conducted upon several questions: 1) Does nutrition have a direct effect on pituitary and ovarian tissues? 2) Does the presence of *PR* receptors in those tissues responsive towards nutritional changes?

In this study, for monitoring progesterone receptor expression, real-time quantitative PCR (RT-qPCR) was used due to its sensitivity as well as accurate quantification (Holzapfel & Wickert, 2007). In the imperative need to obtain expression results that are not only accurate but also reliable, it is important to use specific primer which in this case no specific primer of *PR* gene for pituitary and ovarian tissues of goat established in literature. Thus, new primer was design and optimal standard for gene expression analysis was optimized in this study.

## 1.2 Objective of Study

The objectives of this study are:

1. To determine the effect of short-term concentrate supplementation on ovulation rate of female goat in relation to live weight and body condition.
2. To analyses relationship between size of ovary with number and diameter of CL.
3. To develop RT-qPCR primer for progesterone receptor gene.
4. To determine the effect of short-term concentrate supplementation on expression of progesterone receptor in pituitary and ovary (corpus luteum and follicle) in female goats.

### 1.3 Hypothesis of Study

Hypothesis of this study are:

1. Short-term concentrate supplementation will increase ovulation rate follow by increment in body weight and body condition score of female goats.
2. Bigger ovary size will produce bigger corpus.
3. Specific RT-qPCR primer for progesterone receptor will be developed.
4. Expression of progesterone receptor in pituitary and ovary (corpus luteum and follicle) of supplemented goats will be higher than control goats.



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