



UNIVERSITI PUTRA MALAYSIA

***OVULATION RESPONSES ON THREE OESTRUS SYNCHRONISATION
PROTOCOL AND ASSOCIATION BETWEEN PROGESTERONE
CONCENTRATION IN LATE PREGNANCY AND LITTER SIZE OF BOER
GOATS***

ABDUL MU'IN BIN HASSAN BASRI

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By

ABDUL MU'IN BIN HASSAN BASRI

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

June 2016

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

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Assessment of goat reproductive systems and application of advanced reproductive technologies (ART), help to improve the goat industry in Malaysia. Oestrus synchronisation (OS) protocols and equipment like real-time ultrasonography (RTU) machine are important component of advance reproductive technologies. The objectives of the current study were to determine the ovulation responses on three oestrus synchronisation protocols and association between progesterone levels with litter size in Boer goats. In Experiment 1, twenty-two (24) fertile and healthy Boer does were divided equally into three groups and each group was treated with one of the three treatments:- T1: CIDR for 14 days and 200 IU of pregnant mare serum gonadotrophin (PMSG) and 0.5 ml prostaglandin (PG) prior to CIDR removal; T2: CIDR for 14 days and 0.5 ml PG prior to CIDR removal; T3: CIDR for 9 days and 200 IU of PMSG and 0.5 ml PG prior to CIDR removal. The oestrus sign was observed from 24 hours after CIDR removal to first sign of oestrus. Both ovaries for all does were examined with a RTU machines for 72 hours at six-hours interval beginning at 24 hours after CIDR removal. Blood samples were collected at four points which were:- 24 hours after CIDR removal, at first time of oestrus sign, at first ovulation and at 72 hours after CIDR removal for progesterone hormonal profile assessment. All does demonstrated (100%) oestrus signs but 25%, 12% and 12% in T1, T2 and T3 respectively did not ovulated. Ovulation responses in terms of mean number of follicles observed before first ovulation, mean size of follicles before first ovulation and mean number of follicle ovulated did not show any significant difference ($p > 0.05$) among the T1, T2 and T3. The percentage of does with single and multiple ovulations were not significantly different among the three treatments. However, high percentages of multiple ovulations (83%, 86% and 71%, respectively) were recorded for each T1, T2 and T3 as compared to single ovulation. Mean number of follicles ovulated from right and left ovaries were significantly different ($p < 0.05$) in T3 (2.1 ± 1.0 , 0.6 ± 0.5). The mean time of ovulation were recorded between 44.0 ± 2.85 to 47.4 ± 2.78 hours ($p > 0.05$) among the three treatments

calculated from 24 hours after CIDR removal. All treatments showed no significant difference ($p > 0.05$) for duration of ovulation with 18.0 ± 5.37 , 21.0 ± 5.95 , 19.2 ± 3.98 hours calculated from 24 hour after CIDR removal for T1, T2 and T3 respectively. Assessment of progesterone hormonal profiles at four points did not show any significant difference ($p > 0.05$) among the three treatments. In Experiment 2, 5 ml of blood sample from 48 pregnant does were taken approximately two weeks before expected kidding date. The blood samples were analyzed to determine progesterone hormonal profile. After parturition, the number of litter size for each doe were recorded either single, twins or triplets. The mean progesterone concentration showed no significant difference ($p > 0.05$) that are 17.7 ± 2.9 ng/ml for doe with single kid and 21.4 ± 2.8 ng/ml for does with two or more kids. In conclusion, in Experiment 1, the result of ovulation responses showed no significant differences between Treatment 1 (T1) which was insertion of CIDR for 14 days with the injection of 200 IU of PMSG and 0.5 ml PG prior to CIDR removal ; Treatment 2 (T2), insertion of CIDR for 14 days with the injection of 0.5 ml PG prior to CIDR removal and; Treatment 3 (T3), insertion of CIDR for 9 days with the injection of 200 IU of PMSG and 0.5 ml PG prior to CIDR removal. Meanwhile, in Experiment 2, the high number of litter size did not increase the level of progesterone concentration two weeks before kidding.

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

**TINDAK BALAS OVULASI TERHADAP TIGA PROTOKOL
PENYELARASAN ESTRUS DAN HUBUNGAN ANTARA KEPEKATAN
PROGESTERON PADA AKHIR KEBUNTINGAN DAN BILANGAN ANAK
KAMBING BOER**

Oleh

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Penilaian sistem pembiakan kambing dan aplikasi teknologi pembiakan maju (ART) membantu dalam meningkatkan industri kambing di Malaysia. Protokol penyelarasan oestrus (OS) dan peralatan berteknologi tinggi seperti mesin ultrasonografi (RTU) adalah komponen penting dalam teknologi pembiakan maju. Objektif kajian semasa adalah untuk menentukan tindak balas ovulasi terhadap tiga protokol penyelarasan oestrus hubung kait antara tahap progesteron dengan bilangan anak terhadap kambing Boer. Dalam Eksperimen 1, sebanyak dua puluh empat (24) ekor kambing Boer yang subur dan sihat dibahagikan sama banyak kepada tiga kumpulan dan setiap kumpulan dirawat dengan salah satu daripada tiga rawatan: - T1: CIDR selama 14 hari dan 200 IU *pregnant mare serum gonadotrophin* (PMSG) dan 0.5 ml prostaglandin (PG) sebelum pengeluaran CIDR; T2: CIDR selama 14 hari dan 0.5 ml PG sebelum pengeluaran CIDR; T3: CIDR selama 9 hari dan 200 IU PMSG dan 0.5 ml PG sebelum pengeluaran CIDR. Tanda-tanda oestrus dipantau bermula 24 jam selepas pengeluaran CIDR sehingga tanda pertama oestrus. Kedua-dua belah ovari diperiksa dengan mesin RTU selama 48 jam pada selang enam jam bermula pada 24 jam selepas pengeluaran CIDR. Sampel darah diambil di empat titik iaitu: - 24 jam selepas pengeluaran CIDR, pada masa tanda pertama galak, pada ovulasi pertama dan 72 jam selepas pengeluaran CIDR untuk penilaian profil hormon progesteron. Semua kambing menunjukkan (100%) tanda galak tetapi 25%, 12% dan 12% ini tidak berovulasi untuk T1, T2 dan T3 masing-masing. Tindak balas ovulasi seperti bilangan folikel sebelum ovulasi pertama, saiz folikel sebelum ovulasi pertama dan bilangan folikel terovulasi tidak menunjukkan perbezaan yang ketara ($p > 0.05$) di antara T1, T2 dan T3. Peratusan kambing dengan ovulasi tunggal dan berganda tidak menunjukkan perbezaan ketara antara tiga rawatan. Walau bagaimanapun, peratusan yang tinggi ovulasi berganda (83%, 86% dan 71%) dicatatkan bagi setiap T1, T2 dan T3 berbanding ovulasi tunggal. Bilangan folikel yang mengalami ovulasi pada ovari kanan dan kiri telah menunjukkan perbezaan

yang ketara ($p < 0.05$) pada T3 (2.1 ± 1.0 , 0.6 ± 0.5). Masa ovulasi telah direkodkan di antara 44.0 ± 2.85 - 47.4 ± 2.78 jam ($p > 0.05$) antara tiga rawatan dikira daripada 24 jam selepas pengeluaran CIDR. Semua rawatan tidak menunjukkan perbezaan yang ketara ($p > 0.05$) bagi tempoh ovulasi dengan 18.0 ± 5.37 , 21.0 ± 5.95 , 19.2 ± 3.98 jam dikira dari 24 jam selepas pengeluaran CIDR untuk T1, T2 dan T3. Penilaian untuk profil hormon progesteron di empat titik masa tidak menunjukkan sebarang perbezaan yang ketara ($p > 0.05$) antara tiga rawatan. Dalam Eksperimen 2, 5 ml sampel darah 48 ekor kambing hamil diambil kira-kira dua minggu sebelum tarikh jangkaan kelahiran. Sampel darah dianalisis untuk penentuan profil hormon progesteron. Selepas kelahiran, bilangan anak bagi setiap kambing telah direkodkan sama ada satu, kembar atau triplet. Kepekatan progesteron menunjukkan perbezaan yang ketara ($p > 0.05$) iaitu 17.75 ± 2.9 ng / ml untuk betina dengan anak tunggal dan 21.48 ± 2.8 ng / ml untuk betina dengan dua atau lebih anak. Kesimpulannya, dalam Eksperimen 1, hasil daripada tindak balas ovulasi tidak menunjukkan perbezaan yang signifikan antara Rawatan 1 (T1) yang memasukkan CIDR selama 14 hari dengan suntikan sebanyak 200 IU PMSG dan 0.5 ml PG sebelum pengeluaran CIDR; Rawatan 2 (T2), memasukkan CIDR selama 14 hari dengan suntikan sebanyak 0.5 ml PG sebelum pengeluaran CIDR dan; Rawatan 3 (T3), memasukkan CIDR selama 9 hari dengan suntikan sebanyak 200 IU PMSG dan 0.5 ml PG sebelum pengeluaran CIDR. Sementara itu, dalam Eksperimen 2, peningkatan bilangan anak tidak meningkatkan tahap progesteron dalam dua minggu sebelum kelahiran.

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I certify that a Thesis Examination Committee has met on 21 June 2016 to conduct the final examination of Abdul Mu'in bin Hassan Basri on his thesis entitled "Ovulation Responses on Three Oestrus Synchronisation Protocol and Association between Progesterone Concentration in Late Pregnancy and Litter Size of Boer 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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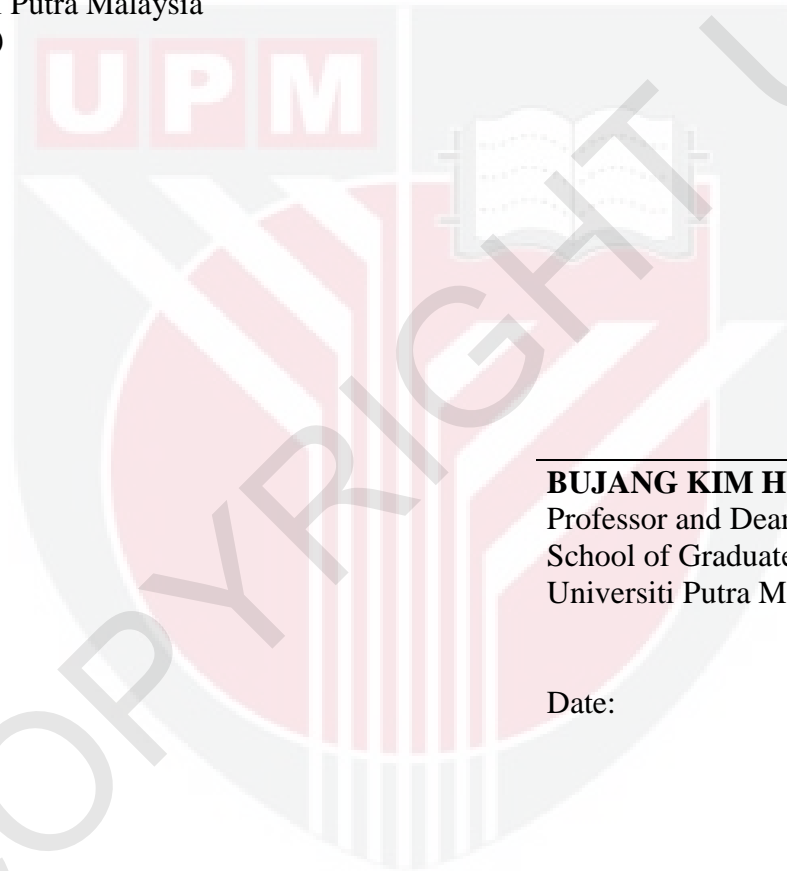
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LIST OF ABBREVIATIONS

AI	Artificial insemination
ANOVA	Analysis of variance
ART	Advance reproductive technologies
CIDR	Controlled internal drug release
D	Day
Ecg	Equine chorionic gonadotrophin
ELISA	Enzyme-linked immunosorbent assay
ET	Embryo transfer
FGA	Flurogestone acetate
FSH	Follicle stimulating hormone
GLM	General Linear Model
GnRH	Gonadotrophin-releasing hormone
i.m	Intra muscular
IU	International Unit
LH	Luteinizing hormone
LH-RH	Luteinizing hormone-releasing hormone
MAP	Methyl acetate progesterone
NS	Non significant
OS	Oestrus synchronisation
Pg	Picogram
PG/ PGF _{2α}	Prostaglandin
PMSG	Pregnant mare serum gonadotrophin
RIA	Radioimmunoassay
Rpm	Round per minute
RTU	Real-time ultrasonography
SE	Standard error
TMB	Tetramethylbenzidine

CHAPTER 1

INTRODUCTION

Goat population in Malaysia has increased from 429,398 in 2014 to 439,667 in 2015 (DVS, 2016). As an alternative to beef as a source of red meat, the increase in goat production is very important to meet the market requirement in the future. The self-sufficiency level for mutton and chevon was estimated to be about 13% in 2014, and for beef, it was about 25%. The increase in goat population in Malaysia is mainly due to the establishment of many local breeding farms. These breeding farms usually import purebred goats and serve as multiplier farms, adopting various breeding strategies and advance reproductive technologies (ART) such as oestrus synchronisation (OS) and artificial insemination (AI).

1.1 Research problem

The government of Malaysia through DVS and Malaysian Agriculture Research and Development Institute (MARDI) has imported many breeding animals of good genetics as well as semen in the effort to improve goat population in Malaysia (Wan Zahari *et al.*, 2008). However, livestock farmers, especially owners of breeding farms, and goat industry workers should have knowledge on reproduction parameters to gain worthy outcomes from the imported breeders and semen. Assessment of reproduction parameters either in male or female goats will help to monitor performance and fertility of goats as well as boost goat production. Reproduction parameters in female goats include the length of oestrous cycle, time of oestrus onset, ovulation time, ovulation rate, conception and pregnancy rates, litter size and reproductive hormonal profiles. Farmers who possess good understanding and skills to assess these reproductive parameters will be able to conduct a good breeding program and save the cost, by successfully identifying good breeding animals and using various reproduction technologies and tools efficiently and effectively. Most of breeding farms in Malaysia used long duration of oestrus synchronisation protocols, and short duration was introduced recently to shorten the cycle. Both treatment were gave good result in term of oestrus response (Abdul Muin *et al.*, 2013). However, there still few works were conducted to assess ovulation response after synchronization in Malaysia to determine the most suitable OS protocol and help to facilitate AI.

In addition to the above, reproductive hormonal profiles can help to determine the number of fetus and to overcome the problem of fetus abortion during late pregnancy that was unofficially reported in some breeding farms. Thus, this study was conducted to determine the effect of oestrus synchronisation on ovulation responses, and to evaluate the association between progesterone concentrations and litter size at late pregnancy in Boer goats.

1.2 Justification

The outcome of this study will provide better information on oestrous cycle in Boer goats under Malaysian conditions. In addition, the information will provide the most suitable and efficient oestrus synchronisation protocol for reproductive logistics. This will facilitate goat breeding via natural or artificial insemination and reduce kidding interval. Farm management could also estimate suitable time to conduct artificial insemination to save the cost of breeding program and to get efficient result. Furthermore, the assessment of goat reproductive hormonal profile will be used as a mean to estimate the number of kid born and help to determine the problem of fetus abortion at late pregnancy.

1.3 Research hypothesis

1. The oestrus synchronisation protocol with a shorter duration of CIDR treatment together with PMSG and PG treatment will give similar or even better response of ovulation as compared to oestrus synchronisation protocol with a long duration of CIDR treatment. Similar or better response would also be expected with shorter duration of the combined hormone treatment compared to longer CIDR treatment with PG (without PMSG).
2. A high number of litter sizes will result in high progesterone (P4) concentrations two weeks prior to kidding.

1.4 Objectives

The general objective of this study was to determine the ovulation responses to different oestrus synchronisation protocols and the association between progesterone (P4) concentrations during late pregnancy and litter size in Boer goat in Malaysia.

The specific objectives were:

1. To evaluate the number of follicles ovulated from three oestrus synchronisation protocols using Controlled Internal Drugs Release (CIDR)
2. To determine the time and duration of ovulation.
3. To determine the P4 and oestrogen (E2) concentrations during oestrus.
4. To investigate the association of P4 concentration at late pregnancy and litter size.

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Abdul Mu'in Bin Hassan Basri was born in Kuala Lipis, Pahang on 20 July 1988. As the third child in a family of six siblings, he starts his schooling at Sekolah Kebangsaan Benta, Pahang and continued his secondary school at Sekolah Menengah Sains Tengku Abdullah, Raub, Pahang.

His tertiary education began when he pursued his study in Pulau Pinang Matriculation before continued for his first degree in the Faculty of Agriculture, Universiti Putra Malaysia (UPM) in 2007. Consequently, he was conferred the Bachelor of Agriculture (Animal Science) in 2011. Then, on 14th February 2012, he enrolled in the Master of Science (MSc) in Animal Production in the Faculty of Agriculture, UPM.

His first manuscript entitled “. Effect of two CIDR-based oestrus synchronisation protocols on oestrus response in Boer goats” has been published in Malaysian Journal of Animal Science (MJAS) Vol 16 (2), 2013. He also presented the posters entitled “Effect of different oestrus synchronisation protocols in Boer goats (*Capra hircus*)” in The 33rd Malaysian Society Of Animal Production Annual Conference 2012 held in Langkawi, Kedah and “Ovulation response to different oestrus synchronisation protocols on Boer goats” in The 34th Malaysian Society of Animal Production Annual Conference held from 3rd to 5th June 2013, Kuantan, Pahang.

LIST OF PUBLICATIONS

- Abdul Muin, H.B.**, Hasbudie, H.B., Suraya, M. S., Panandam, J. M., Yaakub, H., Theivanai, J., Quaza Nizamuddin, H. N. 2013. Effect of two CIDR-based oestrus synchronisation protocols on oestrus response in Boer goats. *Mal. J. Anim. Sci.* 16(2):29-35.
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