UNIVERSITI PUTRA MALAYSIA

REPRODUCTIVE RESPONSE OF GOATS TO DIFFERENT OESTRUS SYNCHRONISATION PROTOCOLS AND THEIR COMBINATION WITH FIXED TIME ARTIFICIAL INSEMINATION

SURAYA MOHAMAD SALLEH

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By

SURAYA MOHAMAD SALLEH

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REPRODUCTIVE RESPONSE OF GOATS TO DIFFERENT OESTRUS SYNCHRONISATION PROTOCOLS AND THEIR COMBINATION WITH FIXED TIME ARTIFICIAL INSEMINATION

By

SURAYA BINTI MOHAMAD SALLEH

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Chairman: Associate Professor Halimatun Yaakub, PhD

Faculty: Agriculture

There is lack of information on the effects of oestrus synchronisation using Controlled Internal Drug Release (CIDR) insert in goats in Malaysia. Understanding the effect of oestrus synchronisation would enable the use of fixed-time artificial insemination (FTAI), which might increase the efficiency of goat production. This study will determine the most suitable oestrus synchronisation protocol and FTAI to be practiced in goat industries in Malaysia. Two experiments were conducted in this study. In the first experiment, 23 Saanen crossbred does (2 to 4 years old) were divided unequally into 3 groups. The first group received CIDR for 14 days, Pregnant Mare Serum Gonadotrophin (PMSG) and cloprostenol at CIDR removal (CIDR14++; n=8); the second group of does received CIDR for 9 days and PMSG and cloprostenol at CIDR removal (CIDR9++; n=8) and the third group received CIDR for 9 days and cloprostenol only at CIDR removal (CIDR9+; n=7). Oestrus signs were recorded and blood samples for progesterone (P4) and luteinising hormone (LH) analysis were collected for 24 h at 4 h interval, starting from 24 h post CIDR removal. Data on oestrus signs, P4 and LH were analysed using one-way ANOVA. Results showed 100 % of does in group CIDR14++ in oestrus, while the other two groups (CIDR9++ and CIDR9+) showed only 57% and 33 %, respectively. The time from CIDR removal until onset of oestrus in CIDR9+ was significantly longer ($P<0.05$) than CIDR9++ and CIDR14++. The P4 concentration at 24 h post CIDR removals decreased when compared with P4 levels at CIDR removal in all groups. The LH concentration at peak (8.03±2.07 mIU/ml) for CIDR14++ was significantly higher ($P<0.05$) than CIDR9+ (7.32±1.07 mIU/ml) and CIDR9++ (4.94±0.80 mIU/ml). The LH peak occurred at 42.0±1.15, 41.0±1.91 and 43.0±1.00 h post CIDR removal for CIDR14++, CIDR9++ and CIDR9+, respectively. The result from this study suggested that the duration of CIDR treatment could be reduced from 14 to 9 days suitable time to conduct fixed-time artificial insemination (FTAI) in goat is at 55 to 66 h after CIDR removal. In the second experiment, 127 Boer does were divided into three groups and inserted with CIDR intravaginally for 14 (CIDR14++; n=42; CIDR14+; n=42) and 9 days (CIDR9++; n=43). Approximately 0.5 ml PG was administered intramuscularly to all groups at CIDR removal and only groups CIDR14++ and CIDR9++ were administered with 200 IU of PMSG

intramuscularly. Does in each oestrus synchronisation protocol were further randomly divided into two subgroups, go through FTAI at either at 55 or 66 h post CIDR removal. The time of AI was chosen based on the results from experiment 1. Oestrus signs were observed at 4 h intervals and blood samples were collected for P4 and LH determination. The number of does pregnant was determined by ultrasonography at 30 days post AI. Data on oestrus observations, P4 and LH were analysed using Chi-square and GLM by SAS 9.3 software. The percentage of does in oestrus within 24 to 72 h post CIDR removal were significantly higher ($P<0.05$) in groups CIDR14++ (97.6%) and CIDR 9++ (100%) compared to group CIDR14+ (81.0%). The numbers of does displaying oestrus signs within 24 to 28 h post CIDR removal were significantly higher ($P<0.05$) in group CIDR9++ compared to groups CIDR14++ and CIDR14+. The P4 concentrations at 24 hours post CIDR removals and LH concentration were not significantly different ($P>0.05$) in all groups. The time of LH peak in group CIDR14+ was significantly delayed ($P<0.05$) when compared to group CIDR9++. The pregnancy rate of FTAI does was not significantly different ($P<0.05$). In conclusion, the suitable oestrus synchronisation protocols for goat in Malaysia were CIDR14++ and CIDR9++. It is recommended to use the treatment CIDR9++, since the oestrous cycle can be shortened. Since the pregnancy rate in this study is very low; the suitable time to conduct fixed-time AI cannot be concluded.

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

RESPONS REPRODUKTIF KAMBING KEPADA PROTOKOL SINKRONISASI ESTRUS YANG BERBEZA DAN KOMBINASI MEREKA DENGAN PERMANIAN BERADAS MASA TETAP

Oleh

SURAYA BINTI MOHAMAD SALLEH

Mac 2014

Pengerusi: Professor Madya Halimatun Yaakub, PhD

Fakulti: Pertanian

Terdapat kekurangan maklumat mengenai kesan sinkronisasi estrus menggunakan *Controlled Internal Drug Release* (CIDR) pada kambing di Malaysia. Pemahaman kesan sinkronisasi estrus akan membolehkan penggunaan permanian beradas pada masa tetap (FTAI), dimana ini mungkin akan dapat meningkatkan efisiensi pengeluaran kambing. Kajian ini akan menentukan sinkronisasi estrus yang paling sesuai dan FTAI di amalkan dalam industri kambing di Malaysia. Dua eksperimen telah dijalankan dalam dalam kajian ini. Dalam eksperimen pertama, 23 ekor kambing Saanen kacukan betina (umur 2 hingga 4 tahun) telah dibahagikan kepada 3 kumpulan. Kumpulan pertama menerima CIDR selama 14 hari, *Pregnant Mare Serum Gonadotrophin* (PMSG) dan *cloprostenol* pada waktu penyingkiran CIDR (CIDR14++; n=8); kumpulan kedua menerima CIDR selama 9 hari, PMSG dan PG pada waktu penyingkiran CIDR (CIDR9++; n=8) dan kumpulan ketiga menerima CIDR selama 9 hari dan hanya PG pada waktu penyingkiran CIDR (CIDR9+; n=7). Tanda estrus telah direkodkan dan sampel darah untuk progesteron (P4) dan luteinising hormon (LH) di ambil untuk 24 jam setiap 4 jam jarak masa bermula 24 jam selepas penyingkiran CIDR. Data tanda estrus, P4 dan LH telah dianalisis menggunakan ANOVA sehala. Keputusan menunjukkan 100 % kambing betina daripada kumpulan CIDR14++ mempunyai tanda estrus, manakala dua kumpulan (CIDR9++ dan CIDR9+) menunjukkan hanya 57% dan 33%, masing-masing. Kepekatan P4 selepas 24 jam penyingkiran CIDR adalah menurun apabila dibandingkan dengan paras P4 untuk semua kumpulan. Kepekatan LH puncak (8.03±2.07 mIU/ml) untuk kumpulan CIDR14++ mempunyai perbezaan bererti lebih tinggi ($P<0.05$) berbanding dengan kumpulan CIDR9+ (7.32±1.07 mIU/ml) dan kumpulan CIDR9++ (4.94±0.80 mIU/ml). Puncak LH berlaku pada 42.0±1.15, 41.0±1.91 and 43.0±1.00 jam selepas penyingkiran untuk kumpulan CIDR14++, CIDR9++ dan CIDR9+, masing-masing. Keputusan experimen ini mencadangkan bahawa jangkamasa rawatan CIDR boleh dikurangkan daripada 14 ke 9 hari dan masa yang sesuai untuk menjalankan permanian beradas adalah pada 55 ke 66 jam selepas penyingkiran CIDR. Dalam eksperimen yang kedua, 127 ekor kambing Boer

betina telah dibahagikan kepada tiga kumpulan dan telah dimasukkan dengan CIDR dalam vagina untuk 14 hari (CIDR14++; n=42; CIDR14+; n=42) dan 9 hari (CIDR9++; n=43). Kira-kira 0.5 ml PG telah disuntik dalam otot untuk semua kumpulan pada penyingkiran CIDR, dan hanya kumpulan CIDR14++ and CIDR9++ menerima suntikan PMSG sebanyak 200 IU of PMSG dalam otot. Kambing betina dalam setiap protocol sinkronisasi estrus akan dibahagikan lagi kepada dua subkumpulan, samada melalui FTAI pada 55 atau 66 jam selepas penyingkiran CIDR. Masa untuk pemanian beradas dipilih adalah berdasarkan keputusan dalam eksperimen 1. Tanda estrus telah direkodkan dan sampel darah telah diambil untuk pada setiap 4 jam jarak masa untuk penentuan P4 dan LH. Bilangan kambing betina bunting ditentukan pada hari 30 selepas AI. Data tanda estrus, P4 dan LH dianalisis menggunakan Khi-kuasadua dan GLM dengan perisisian SAS 9.3. Peratus kambing betina dengan estrus dalam jangkamasa 24 hingga 72 jam selepas penyingkiran CIDR mempunyai perbezaan bererti tinggi ($P<0.05$) dalam kumpulan CIDR14++ (97.6%) dan CIDR 9++ (100%) berbanding kepada kumpulan CIDR14+ (81.0%). Bilangan kambing betina yang menunjukan tanda estrus dalam jangkamasa 24 hingga 28 jam selepas penyingkiran mempunyai berbezaan bererti tinggi ($P<0.05$) dalam kumpulan CIDR 9++ berbanding kepada kumpulan CIDR14++ dan CIDR14+. Kepekatan P4 pada 24 jam selepas penyingkiran CIDR dan kepekatan LH tiada perbezaan bereti ($P>0.05$) dalam semua kumpulan. Masa LH puncak dalam kumpulan CIDR14+ mempunyai berpezaan bererti lambat ($P<0.05$) apabila dibandingkan kepada kumpulan CIDR9++. Kadar kebuntingan FTAI kambing betina adalah tiada perbezaan bererti ($P<0.05$). Untuk kesimpulan, protocol sinkronisasai estrus yang sesuai untuk kambing di Malaysia adalah CIDR14++ and CIDR9++. Ia dicadangkan untuk menggunakan rawatan CIDR9++, memandangkan kitaran estrus boleh dipendekkan. Memandangkan kadar kebuntingan dalam kajian ini adalah rendah, kesesuaian masa untuk menjalankan pemanian beradas pada masa tetap tidak boleh dibuat kesimpulan.

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I certify that a Thesis Examination Committee has met on 28 March 2014 to conduct the final examination of Suraya Mohamad Salleh on her thesis entitled "Different Oestrus Synchronisation Protocols and Fixed Time Artificial Insemination 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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Universiti Putra Malaysia
(Internal Examiner)

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(Internal Examiner)

Wan Khadijah Wan Embong, PhD
Professor
Faculty of Science
Universiti Malaya
Malaysia
(External Examiner)

_____

(Noritah Omar, PhD)
Professor and Deputy Dean
School of Graduate Studies
Universiti Putra Malaysia

Date:

This thesis was submitted to the Senate of Universiti Putra Malaysia and has been accepted as fulfilment of the requirement for the degree of Master of Science. The members of the Supervisory Committee are as follows:

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

Jothi Malar Pananadam, PhD
Professor
Faculty of Agriculture
Universiti Putra Malaysia
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LIST OF ABBREVIATIONS

AI	Artificial Insemination
ANOVA	Analysis of Variance
CIDR	Controlled internal drug release
DMRT	Duncan Multiple Range Test
DVS	Department of Veterinary Services
E2	Oestrogen
eCG	equine Chorionic Gonadotrophin
EIA	Enzyme Immunoassay
ELISA	Enzyme Linked Immunosorbent Assay
ET	Embryo Transfer
FGA	Fluorogestone Acetate
FSH	Follicle Stimulating Hormone
FTAI	Fixed-time Artificial Insemination
GLM	General Linear Model
HRP	Horseradish Peroxidase
LH	Luteinising Hormone
MAP	Medroxyprogesterone Acetate
MGA	Melengesterol Acetate
OS	Oestrus Synchronisation
P4	Progesterone
PD	Pregnancy Diagnosis
PG	Prostaglandin
PGF2α	Prostaglandin F2 alpha

PMSG	Pregnant Mare Serum Gonadotrophin
PRID	Progesterone-releasing intravaginal device
RIA	Radioimmunoassay
SAS	Statistical Analysis System
SE	Standard Error

CHAPTER 1

INTRODUCTION

The goat population in Malaysia was estimated by the Department of Veterinary Services (DVS) as 545,682 in 2010, with only 11.28% self-sufficiency for mutton/chevon (DVS, 2010). Productivity of a farm depends on the overall management of the farm and the herd. One of the important factors in managing a farm is management of the breeding stock. Improvement of the productivity of the goat industry and mutton/chevon self-sufficiency requires genetic improvement of the goats. Reproductive technologies such as oestrus synchronisation, artificial insemination (AI), superovulation and embryo transfer have been used since the 60s to increase the productivity of a farm. AI enables superior genetic materials to be disseminated widely, stored or even manipulated, and reduces the risk of spreading sexually transmitted diseases (Leboeuf *et al.,* 2003). In addition, AI, as with superovulation and embryo transfer, it becomes more efficient only if oestrus synchronisation is practised.

Synchronisation of oestrus in small ruminants has been established since the 1960 in sheep and 1970 in cattle. There are several intravaginal devices that have been used in small ruminants, including progesterone-releasing intravaginal device (PRID) and controlled internal drug release (CIDR) inserts. Nowadays, several studies are being conducted to shorten the duration of oestrus synchronisation so as to minimise the cost of this program. The use of several hormones in oestrus synchronisation also can increase the pregnancy rate as well as the prolificacy rate (Charray *et al.,* 1992).

For AI to be successful following oestrus synchronisation, the timing of insemination should be impeccable. Oestrus detection in the FTAI is not necessary and it can reduce labour cost. Fixed-time AI (FTAI) protocols have been developed for cattle (Geary, 2001; Bader *et al.,* 2005; Perry and Perry, 2008) that allow AI without the need of oestrus detection and have resulted in high success rate.

Understanding the effects of oestrus synchronisation using CIDR in goats would enable the use of FTAI, which would in turn increase the efficiency of AI, subsequently improving the local goat production. Fixed-time AI is dependent on the time of ovulation. Ovulation takes place towards the end of the oestrus period, and this is very important for the sperm to meet and fertilize the ovum. Thus, accurate determination of ovulation time and prediction of the optimal time to inseminate the does are vital for the success of any AI programs. Therefore, the hormonal profiles of progesterone and luteinising hormone during oestrus are vital.

Significance of Study

In Malaysia, there is lack of information on the effects of oestrus synchronisation using CIDR in goats. The use of FTAI would increase the efficiency of meat or dairy goat production, by reducing the problems associated with oestrus detection and cost of AI service, and enabling the transfer of the best quality genetic material.
Thus, this study determines the suitable oestrus synchronisation protocol and the right time to do artificial insemination under Malaysian conditions.

Objectives

The general objective of the present study is to determine the suitable oestrus synchronisation protocol and select the time for fixed-time artificial insemination (FTAI) in goats.

Thus, the specific objectives are:

1) To compare three oestrus synchronisation protocols using CIDR in Saanen crossbred and Boer goats.
2) To profile the progesterone and luteinising hormone concentrations in oestrus synchronized Saanen crossbreds and Boer goats.
3) To evaluate the pregnancy rate of the combined effects of three synchronisation protocols and two fixed-time AI.

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