UNIVERSITI PUTRA MALAYSIA

EQUINE CHORIONIC GONADOTROPHIN INFLUENCES ON FOLLICULAR DEVELOPMENT, OVULATION RATE AND GESTATIONAL DEVELOPMENT IN CROSS-BRED GOATS

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MOHAMAD SHUIB MOHAMAD MOHTAR

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

July 2012

DEDICATION

I dedicate this thesis to my inspiring mom, Noriah Abu Hassan and dad, Mohd Mohtar Yusup, who wanted me to be a role model in the family, and now your wishes come true.

And to my wife, Suhaini Mohd Salleh and precious children, Nor Fatiha Insyirah & Muhamad Faris Amsyar

May this thesis be your inspiration for your future endeavors…

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

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Chair: Professor Abd Wahid Haron, PhD

Faculty: Veterinary Medicine

Superovulation techniques are usually conducted to enhance ovarian follicular activity and subsequently increase reproductive efficiency in goat breeding. This study was carried out to depict the effects of a superovulation technique using equine chorionic gonadothrophin (eCG) treatment on increasing follicular growth, ovulation rate and numbers of kid born. In this study, 29 non-pregnant pluriparous Boer does weighing between 45 and 65 kg, with good body condition were randomly selected and allocated into 3 groups, namely Group 1 (G1, n=11), Group 2 (G2, n=8) and Group 3 (G3, n=10). All does were synchronized using CIDR containing 0.3 g progesterone intravaginally for 18 days. Blood samples from every doe were

collected from the jugular vein using test tubes containing heparin during CIDR treatment (Day 0, 6, 13, 18) and post CIDR treatment (Day 19, 20, 27, 34, 41 and 48). On Day 17 of the CIDR treatment, superovulation treatment using eCG was given intramuscularly using three different treatment dosages. Does in G1, G2 and G3 received 600 IU, 800 IU and 1000 IU eCG, respectively on the same days. Follicular identification on the left and right ovary was conducted using transrectal ultrasonography technique for 4 consecutive days starting on Day 17 of the CIDR treatment. After CIDR removal, oestrus responses in all does were recorded and natural mating was conducted. After 7 days post-mating, the transrectal ultrasonography technique was applied to identify the numbers of corpora lutea present in each side of the ovary. Subsequently, pregnancy diagnosis was carried out using transrectal ultrasonography technique at 30 days post-mating. Measurements of sizes of placentomes were carried out using transrectal ultrasonography on Day 42, 49, 56 and 63 of pregnancy. Data of does kidded were collected after the pregnancy period. Result of the study showed that the range of percentage of does showing oestrus within 48 h after CIDR removal in the three groups was 62.5% to 81.8%. The G1 does (54.5%) had a significantly higher ($P< 0.05$) compared to G2 (25%) and G3 (20%) oestrus response within 24 h after CIDR removal. No significant differences among treatments were observed in terms of the number of follicles developed except for small sized follicles on Day 19 and 20. Meanwhile, the G2 does had the highest ovulation rate ($P< 0.05$) of 2.38 ± 0.32 CL compared to G3 (1.6 ± 0.22 CL) and G1 (1.45 ± 0.15 CL). The average pregnancy rate showed 11 out of 21 oestrus does were pregnant (52.4%) with G3 does (71.4%) having the highest pregnancy rate

compared to the other groups at 55.5% (G1) and 20% (G2). Based on progesterone concentration analysis, all treatments does except unsuccessful mated does in G3 showed increasing level of progesterone starting on Day 0 (CIDR inserted) until next 6 days. After Day 6, the progesterone level for all treatments does have continuously decline until Day 18 (CIDR removal), 19 and lastly Day 20. Only after Day 20, the progesterone level was tremendously increased and maintains above 20 ng/ml until 30 days pregnancy in successful mated does. Whereas, for unsuccessful mated does their progesterone level was peak on Day 34 before decline lower than 5 ng/ml until Day 48. Significant differences in the progesterone level were observed between pregnant and non-pregnant does at Day 41 pregnancies for G1 and at Day 48 for G2 and G3 does post-CIDR insertion. No significant differences were observed among treatments in terms of the size of placentomes (in early stage of pregnancy), kidding rate and gestation length. In conclusion, superovulation by eCG with a long term progesterone treatment in goats has increased the ovulatory activity to produce more matured follicles, high oestrous response and twinning rate. However, this thesis also revealed that the growth of placentome and gestation length were not affected by the different dosages of eCG.

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

PENGARUH GONADOTROPHIN KORIONIK EKUIN KEATAS PERKEMBANGAN FOLIKEL, KADAR OVULASI DAN PEMBANGUNAN KEBUNTINGAN PADA KAMBING KACUKAN

Oleh

MOHAMAD SHUIB MOHAMAD MOHTAR

Julai 2012

Pengerusi: Profesor Abd Wahid Haron, PhD

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Teknik superovulasi kebiasaannya dilaksana bagi meningkatkan aktiviti perkembangan folikel dan pada masa yang sama meningkatkan kecekapan pembiakan pada kambing. Kajian ini dilaksanakan bagi memperlihatkan kesan-kesan teknik superovulasi dengan menggunakan rawatan Gonadotrophin Korionik ekuin (eCG) keatas peningkatan pertumbuhan folikel, kadar ovulasi dan bilangan anak dilahirkan. Di dalam kajian ini, 29 ekor kambing betina “pluriparous” Boer dengan berat badan diantara 45 dan 65 kg, beserta kondisi badan yang baik dipilih secara rambang dan ditempatkan kepada 3 kumpulan, iaitu Kumpulan 1 (G1, n=11), Kumpulan 2 (G2, n=8) dan Kumpulan 3 (G3, n=10). Kesemua kambing telah

disinkroni menggunakan CIDR yang mengandungi 0.3 g progesteron ke dalam vagina selama 18 hari. Sampel darah daripada kambing akan diambil daripada vena jugular menggunakan tabung uji yang mengandungi heparin semasa rawatan CIDR (hari 0, 6, 13, 18) dan selepas rawatan CIDR (hari 19, 20, 27, 34, 41 dan 48). Pada hari 17 rawatan CIDR, rawatan superovulasi menggunakan eCG diberi melalui intra-otot menggunakan tiga rawatan dos yang berbeza. Kambing betina G1, G2 dan G3 masing-masing akan menerima 600 IU, 800 IU dan 1000 IU eCG pada hari yang sama. Pengenalpastian folikel di kiri dan kanan ovari telah dilaksana menggunakan teknik transrektum pengimbasan ultra bunyi pada 4 hari berturut-turut bermula pada hari 17 rawatan CIDR. Selepas CIDR dikeluarkan, reaksi estrus pada semua kambing betina direkod dan pembiakan semulajadi dilaksanakan. Tujuh hari selepas dikahwinkan, teknik transrektum pengimbasan ultra bunyi telah digunakan bagi mengenalpasti bilangan korpus luteum yang hadir di setiap belah ovari. Berikutnya, diagnosa kebuntingan telah dilaksanakan menggunakan teknik transrektum pengimbasan ultra bunyi pada hari ke 30 selepas dikahwinkan. Pengukuran saiz placentom telah dilaksana menggunakan teknik transrektum pengimbasan ultra bunyi pada hari 42, 49, 56 dan 63 kebuntingan. Data kambing betina beranak dikumpul selepas tempoh kebuntingan. Keputusan kajian menunjukkan julat peratusan kambing betina yang menunjukkan estrus dalam lingkungan 48 jam selepas CIDR dikeluarkan pada ketiga-tiga kumpulan ialah 62.5% hingga 81.8%. Kambing-kambing G1 (54.5%) mencatat peratus tertinggi yang ketara ($P< 0.05$) apabila dibandingkan dengan G2 (25%) dan G3 (20%) reaksi estrus dalam lingkungan 24 jam selepas CIDR dikeluarkan. Tiada perbezaan ketara diantara rawatan-rawatan

tersebut merujuk kepada bilangan folikel terhasil kecuali bagi saiz folikel kecil pada hari 19 dan 20. Sementara itu, kambing-kambing G2 mempunyai kadar ovulasi tertinggi ($P< 0.05$) iaitu 2.38 ± 0.32 CL berbanding G3 (1.6 ± 0.22) dan G1 (1.45 ± 0.15). Purata kadar kebuntingan menunjukkan 11 daripada 21 kambing yang estrus telah bunting (52.4%) dengan G3 (71.4%) mencatatkan kadar kebuntingan tertinggi berbanding kumpulan-kumpulan lain pada 55.5% (G1) dan 20% (G2). Berdasarkan analisa kepekatan progesteron, kesemua kambing terawat kecuali kambing yang tidak berjaya kahwin di G3 telah menunjukkan peningkatan paras progesteron bermula pada hari 0 (CIDR dimasukkan) sehingga 6 hari kemudian. Selepas hari 6, paras progesteron untuk kesemua kambing-kambing terawat telah turun berterusan sehingga hari 18 (CIDR dikeluarkan), 19, dan akhirnya hari 20. Selepas hanya hari 20, tahap progesteron telah mendadak naik dan kekal diatas 20 ng/ml sehingga 30 hari kebuntingan bagi kambing-kambing yang berjaya dikahwinkan. Disebalik itu, bagi kambing-kambing yang gagal dikahwinkan paras progesteron ia telah memuncak pada hari 34 sebelum turun serendah daripada 5 ng/ml sehingga hari 48. Tahap progesteron yang berbeza ketara telah dilihat diantara kambing bunting dan tidak bunting di hari 41 kebuntingan bagi G1 dan pada hari 48 bagi kambing-kambing G2 dan G3 selepas CIDR dimasukkan. Tiada perbezaan bererti dilihat diantara rawatan-rawatan pada saiz plasentom (pada peringkat awal kebuntingan), kadar kelahiran dan tempoh kebuntingan. Kesimpulannya, superovulasi menggunakan eCG bersama satu tempoh rawatan progesteron yang panjang pada kambing telah meningkatkan aktiviti ovari bagi menghasilkan lebih banyak folikel matang, reaksi estrus yang tinggi dan kadar kembar. Walau bagaimanapun, kajian ini

turut mendedahkan pertumbuhan plasentom dan tempoh kebuntingan adalah tidak dipengaruhi oleh perbezaan dos-dos eCG.

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I certify that a Thesis Examination Committee has met on 26 July 2012 to conduct the final examination of Mohamad Shuib Mohamad Mohtar on his thesis entitled "Equine Chorionic Gonadotrophin Influences on Follicular Development, Ovulation Rate and Gestational Development in Cross-bred 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 degree of Master of Science.

Members of the Thesis Examination Committee were as follows:

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

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Date:

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 is not concurrently, submitted for any other degree at Universiti Putra Malaysia or at any other institutions.

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MOHAMAD SHUIB MOHAMAD MOHTAR

Date: 26 July 2012.

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ADG	average daily gain
AI	artificial insemination
ANOVA	analysis of variance
AV	artificial vagina
BC	before century
BE	Bailey ejaculator
BSE	breeding soundness examination
CASA	computer assisted sperm analysis
CIDR	Controlled Internal Drug Release
CL	corpus luteum
CV	coefficient of variance
D	day
DVS	Department of Veterinary Services
eCG	equine Chorionic Gonadotrophin
EE	electro-ejaculation
ET	embryo transfer
FCR	feed conversion ratio
FELDA	Federal Land Development Authority
FGA	fluorogestone acetate
FSH	follicle stimulating hormone
FSH-P	follicle stimulating hormone based porcine origin
G	force of gravity
g	gram
GnRH	gonadotropin releasing hormone
h	hours

HAP	horse anterior pituitary extract
IVF	in vitro fertilization
i.m	intramuscular
LH	luteinizing hormone
LOPU	laparoscopic ovum pick-up
MHz	Megahertz
MOA	Ministry of Agriculture and Agro-based Industry of Malaysia
MOET	multiple ovulation and embryo transfer
MPA	medroxyprogesterone acetate
MT	metric tonne
NSB	non-specific binding
OPU	ovum pick-up
PASW®	predictive analytics software
PD	pregnancy diagnosis
PGF2α	prostaglandin F2α
PMSG	pregnant mare serum gonadotrophin
PS	placentome size
P_4	progesterone
pFSH	follicle stimulating hormone based porcine origin
QC-M	quality control medium pool
RIA	radioimmunoassay
r	correlation
r^2	regression
S.E.M	standard error of the mean
µg	microgram

CHAPTER 1

INTRODUCTION

The purpose of goat (*Capra hircus*) rearing is much the same as the purpose of other ruminant species, which is to take advantage of the available forage in order to produce products that humans can consume. Apart from meat and milk as the main products, goats also provide leather to produce shoes, bag, water containers besides wool for clothing, and dung as fertilizers and fuel. They can also be used as a means of entertainment and religious sacrifice. In recent years, various goat parts have been utilized by manufacturing and pharmaceutical companies for the production of gelatin, glue and other products. Exactly when the individual animal species were domesticated is unknown. However, the earliest domestic food species (as most Westerners currently define it) was sheep (somewhere around 8000 BC), followed closely by goats, hogs and cattle (6500 BC), ilama (4000 BC), donkey (3500 BC) and chickens (3200 BC) (Stephen, 2003). Only a few decades ago more attention has been given by scientists and animal producers to the goat species. As with all other species, the genetics and the environment of the goat determine its performance. It is the job of the person (the breeder) in charge of the selection program to combine the best set of genes available for the environment in which the animal will be produced. The challenge of the selection is to improve the genetic potential of the next generation. Improving the ability of a species to produce a needed product in an economically beneficial way requires more research. Selection and breeding

programs for goats can have rather narrow goals, such as high daily weight gain and improvement of quality of carcass.

For most commercial goat producers, crossbreeding is the method of choice for a breeding program. The reason is simply that crossbred goats perform better than purebred goats in meat production. A crossbreeding program for goats is fairly easy to design, and a good variety of productive, adaptable breeds of goats from which to select is available. Crossbreeding is practised when the benefits of heterosis are desired in the offspring. In goats, this approach generally involves crossing bucks of the meat-type sire breeds with does of the fecund-type dam breeds to produce kids with increased growth rate and carcass quality while benefiting from the productive rate and maternal influence of female parent (Shrestha and Fahmy, 2007). In Australia, Dhanda *et al*. (1999) concluded that although crossbreeds demonstrated an advantage in carcass characteristics, there was no important influence on meat quality and nutritious composition. In crossing between German Fawn and Katjang goats in Malaysia, Hirooka *et al.* (1997) reported significant direct and maternal additive genetic effects for body weights at birth, 6 and 9 months of age, suggesting a large difference in the growth rate. Most crossbreeding is done within the confines of a system designed to maximize diverse and complementary traits in the individual parent breeds.

The Boer goat breed which excels in meat production was developed in the 1920s by South African farmers from the Eastern Cape when indigenous goats kept by the

Hottentot and Bantu tribe were crossed with imported Nubian and Indian goats (Skinner, 1972; Erasmus, 2000; Malan, 2000). If meat production is the goal and good Boer stock is available at reasonable prices, then crossbreeding and breeding-up could easily be practised to produce superior meat-type goats. These programs together with selection are required to genetically characterize local goat breeds as well as the exotic breeds for genetic improvement (Barilet, 2007; Fahmy and Shrestha, 2000; Shrestha and Fahmy, 2007).

The goat could only contribute approximately to 2,390 tonnes of meat compared with the consumption demand of 22,550 tonnes in Malaysia (Malaysia Production Agro-Food Statistics Books, MOA, 2010). The Boer goats were introduced into Malaysia in 2000 because this breed can adapt well to the hot and humid conditions. Boer goats have also a high average daily gain (ADG) at 147.1 g/day (Solaiman *et al*., 2011) and low feed conversion ratio (FCR) compared with other breeds of goats. In addition, the percentage of meat is high with almost 54.1% meat obtained from the carcass (Solaiman *et al*., 2011).

To increase productivity in goats, superovulation is one of the biotechnological tools which can be utilized to increase the number of kids born alive. Other approaches to multiply the number of kids born is through artificial insemination (AI), embryo transfer (ET) and in vitro fertilization (IVF). Superovulation permits multiple number of ovum release from the ovary, so that more ova can be fertilized. For superovulation, three kinds of hormones have been widely used. They are, equine

chorionic gonadothropin (eCG) formerly known as "pregnant mare serum gonadotropin" (PMSG), follicle stimulating hormone (FSH) and horse anterior pituitary (HAP) extract. These hormones are grouped as gonadotropins because they stimulate ovarian follicle growth. They differ among by theirs schedule of treatment, preparation and dosage regimens. Naqvi and Gulyani (1999) indicated that the different gonadotrophin regimens have variable effects on superovulation, ova recovery and the fertilization rate.

The combination of eCG and progestagen treatments eliminates the variability in the ovulatory response of goats both in the breeding season and in the seasonal anoestrus period. This resulted in an increased ovulation rate and fertility improvement (Van der Wethuysen, 1979; Ritar *et al*., 1984). However, the dose of eCG that should be used to increase litter size during the breeding season is still not well defined (Regueiro *et al*., 1999).

The present study aimed to compare the lower dosages of eCG at 600 IU and 800 IU compared to 1000 IU which eventually improve reproductive efficiency as well as reducing cost of superovulatory treatment in the goats.

Therefore, this study was conducted with the following objectives:

i. to compare the effect of different dosages of eCG on oestrus response time.
ii. to analyze the follicle development, ovulation and pregnancy rates.
iii. to compare the size of placentomes, gestation length and kidding rate following different doses of eCG administration.
iv. to compare the effectiveness of low dosages of eCG treatment on the number of kids born per doe.

There are several limitations to this study. They are**:**

i. Small numbers of non-pregnant does with good body condition that were available at the farm.
ii. Inconsistency of feeding times to the animals that produced bad effects to the mating season and fetal development in does.
iii. Less experience on identification of corpora lutea during ultrasound scanning.

The hypothesis of this study is that lower dosages of eCG at 600 IU and 800 IU provide similar effectiveness of superovulation treatment to improve reproductive efficiency in the goats.

CHAPTER 7

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