UNIVERSITI PUTRA MALAYSIA

SUPEROVULATION AND EGG RECOVERY IN GOATS

ROSNINA BTE. HAJI YUSOFF

FPV 1989 2

SUPEROVULATION AND EGG RECOVERY IN GOATS

By

ROSNINA BTE. HAJI YUSOFF

Thesis Submitted in Partial Fulfilment of the
Requirements for the Degree of Master of Science
in the Faculty of Veterinary Medicine and
Animal Science
Universiti Pertanian Malaysia

June 1989

ACKNOWLEDGEMENTS

Praise to the Almighty Allah. Had it not been His Will the completion of this study would not have been possible.

I would like to express my sincere gratitude and appreciation to:

i. Professor M.R. Jainudeen, my supervisor, for his invaluable guidance, advice, encouragement and patience throughout this study.

ii. Dr. Sharifuddin Wahab for his advice, interest and support throughout this study.

iii. Dr. Mohd. Ali Rajion and the staff of U.P.M Goat Unit for their kind cooperation.

iv. Zaid Othman, my husband, for his support, concern, understanding and patience throughout this study.

v. My mother, family and friends for their patience, encouragement and support.

vi. Universiti Pertanian Malaysia for financial support through Project 50303/JO2 IRPA.

TABLE OF CONTENTS

LIST OF TABLES

LIST OF FIGURES

LIST OF PLATES

LIST OF ABBREVIATIONS

CL	=	corpora lutea
cm	=	centimetre
FSH	=	Follicle Stimulating Hormone
h	=	hours
GnRH	=	Gonadotrophin Releasing Hormone
HCG	=	Human Chorionic Gonadotrophin
i.u	=	International Unit
kg	=	kilogram
LH	=	Luteinizing Hormone
LHRH	=	Luteinizing Hormone Releasing Hormone
mcg	=	microgram
mg	=	milligram
ml	=	millilitr
mm	=	millimetre
ng	=	nanogram
pct	=	percent
pg	=	picogram
PGF2 alfa	=	Prostaglandin F2 alfa
PMSG	=	Pregnant Mare Serum Gonadotrophin
rpm	=	revolutions per minute

Abstract of thesis presented to the Senate of Universiti Pertanian Malaysia as partial fulfilment of the requirements for the degree of Master of Science

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ROSNINA BTE. HAJI YUSOFF

June 1989

Supervisor : Professor M. R. Jainudeen
Faculty : Veterinary Medicine and Animal Science

Research on superovulation, egg recovery and embryo transfer in goats has been conducted in many developed countries of the world, but similar studies in tropical goats are limited in South-East Asia. Therefore a study was undertaken to determine if the indigenous goat, Kambing kacang, and their crosses could be superovulated and eggs recovered by techniques previously reported for goats in the temperate zone. A total of 70 Kambing kacang does were superovulated with either Pregnant Mare Serum Gonadotrophin (PMSG) or Follicle Stimulating Hormone (FSH-P). PMSG (500, 1000 and 1500 i.u) was administered intramuscularly as a single dose, two days before the withdrawal of 60 mg medroxyprogesterone acetate intravaginal sponges. FSH-P

(10, 15 and 20 mg) was divided into four decreasing daily doses (4,3,2,1; 6,4,3,2; 8,6,4,2 mg) and administered subcutaneously at 0700 h and 1900 h. The interval from end of superovulatory treatment to the onset of oestrus was longer among the PMSG-treated groups (38 to 63 h) compared with the FSH-treated groups (19 to 39 h). The superovulatory response was less than 50 pct during the hot months of February, June and July when the monthly rainfall was below 100 mm. The average number of unruptured follicles did not vary greatly between and within gonadotrophin-treated groups but the average number of CL was higher in the FSH-treated groups (7 to 10 CL per doe) than in the PMSG-treated groups (1 to 5 CL per doe). However, the average rate of egg recovery and fertilization from both the gonadotrophin-treated groups were poor. From these results it may be concluded that a study on the folliculogenesis of the Kambing kacang does should be conducted to determine the optimum time of gonadotrophin injections so as to improve the superovulatory response. Techniques on recovery of eggs via laparoscopy or via the cervix should also be developed to prevent adhesions and thus, improving greater use of donors.

Abstrak tesis yang dikemukakan kepada Senat Universiti Pertanian Malaysia bagi memenuhi sebahagian daripada syarat-syarat untuk ijazah Master Sains

SUPEROVULASI DAN PENGUMPULAN TELUR DARI KAMBING

Oleh

ROSNINA BTE. HAJI YUSOFF

Jun 1989

Penyelia : Profesor M.R. Jainudeen
Fakulti : Kedoktoran Veterinar dan Sains Peternakan

Penyelidikan terhadap superovulasi, pengumpulan telur dan pemindahan embrio pada kambing telah dilaksanakan di negara-negara yang telah maju tetapi kajian tersebut pada kambing-kambing tropika masih terhad di Asia Tenggara. Dengan itu, suatu kajian telah dilaksanakan bagi menentukan samaada kambing tempatan, Kambing kacang dan kacukannya boleh disuperovulasikan dan telur-telurnya dikumpul mengikut teknik-teknik yang telah dilakukan ke atas kambing-kambing di zon temperate. Sejumlah 70 ekor Kambing kacang betina telah disuperovulasikan dengan "Pregnant Mare Serum Gonadotrophin" (PMSG) atau "Follicle Stimulating Hormone" (FSH-P). PMSG (500, 1000 dan 1500 i.u) disuntik melalui otot untuk satu dos sahaja, dua hari sebelum "sponge" yang mengandungi 60 mg medroxyprogesterone acetate ditarik keluar dari faraj

kambing. FSH-P (10, 15, 20 mg) dibahagi kepada 4 dos secara menurun (4,3,2,1; 6,4,3,2; 8,6,4,2 mg) selama 4 hari dan disuntik di bawah kulit kambing pada jam 0700 dan 1900. Jangkamasa antara akhir rawatan superovulasi dan bermulanya estrus adalah lebih panjang dalam kumpulan PMSG (38 hingga 63 jam) berbanding dengan kumpulan FSH (19 hingga 39 jam). Dalam bulan Februari, Jun dan Julai dimana jumlah hujan adalah kurang dari 100 mm sebulan, tindakbalas superovulasi kurang dari 50 peratus. Purata bilangan folikel yang belum pecah tiada beza antara kedua-dua kumpulan gonadotropin tetapi purata bilangan CL lebih tinggi di kalangan kambing-kambing yang dirawat dengan FSH (7 hingga 10 CL bagi seekor kambing betina) berbanding dengan kumpulan kambing PMSG (1 hingga 5 CL bagi seekor kambing betina). Walau bagaimanapun, purata bagi kadar ova yang telah dikumpul dan disenyawakan dari kedua-dua kumpulan gonadotropin adalah kurang memuaskan. Dari keputusan-keputusan tersebut, satu kajian mengenai pembentukan dan perkembangan folikel (folikulogenesis) Kambing kacang hendaklah dilaksanakan bagi menentukan masa yang optima untuk menyuntik gonadotropin bagi memperbaiki tindakbalas superovulasi. Selanjutnya teknik mengumpul ova secara laparoskopi atau melalui serviks hendaklah diperkembangkan untuk mengelak penaupan tisu dan dengan itu memperbaiki kegunaan kambing-kambing betina.

CHAPTER 1

INTRODUCTION

The location of the world's goat population is towards the equatorial/tropical regions where more than two-thirds are within 30° of the equator. This is because from the biological standpoint, the small size, large surface area relative to body weight and limited subcutaneous fat cover make it poorly adapted to cold climates but relatively well adapted to high temperature. In the arid tropics, it adapts well because of its ability to conserve water, travel well, graze selectively and take a wide variety of vegetation. Moreover, the indigenous goat is resistant to many diseases which plague other livestock species in these areas.

The goat population of Malaysia which comprised mainly of Kambing kacang is reared for meat, which provides smallholder farmers a supplementary income. Between 1980 and 1984, the goat population has declined rapidly from 352,300 to 288,980 (Jabatan Perkhidmatan Haiwan, Malaysia, 1984). This decline in number is due to the increased extraction of goats for meat and, although the indigenous goat breeds throughout the year, two kiddings per year are not common since they exhibit long kidding intervals.

To increase local production so as to meet local demand, the genetic improvement of the indigenous goat is urgently needed. The latest high-technology is to dwell into the manipulation of gametes by embryo transfer technology which include superovulation, collection and transfer of embryos to recipients.

Through embryo transfer, the number of progeny from genetically superior females could be increased which helps to intensify a breeding programme and significantly reduces the time span required to achieve the same outcome by natural breeding. With embryo transfer, the frequency of a particular genetic characteristics within existing populations could be increased. The increased progeny allows more intensive selection for desired traits as well as advancing the overall numbers of superior animals produced in a short period. Eventually, embryo transfer acts as an aid in the transport of animals.

The objectives of this study are:

1. To establish a method of oestrus induction in the indigenous goat, Kambing kacang and its crosses.
2. To compare the superovulatory response to different doses of either Follicle Stimulating Hormone (FSH) or Pregnant Mare Serum Gonadotrophin (PMSG).
3. To adapt a technique for recovery of eggs from the reproductive tract of the superovulated goat.

CHAPTER 2

LITERATURE REVIEW

Introduction

Embryo transfer procedure involves a series of integrated steps: superovulation of donors, synchronisation of oestrus between donors and recipients, recovery, storage and transfer of embryos. The technique of embryo transfer is not only of considerable potential in genetic improvement, but because the number, genotype, age of embryos, factors can be controlled, also has many potential applications in research.

In general, embryo transfer has the benefit of increasing the number of progeny from genetically superior females, increasing particular genetic characteristics within existing populations and acting as a method of transport of animals between countries. For a better understanding of embryo transfer procedure in goats and sheep, pertinent aspects of female reproductive physiology, superovulation and collection of embryos are reviewed in this chapter.

Female Reproduction

Reproduction in the female is a complex phenomenon. It is dependent on various factors such as hereditary characters, endocrine constitution and pathological conditions, nutrition and management. If the physiological processes are in harmony and the above factors are conducive, the female will reach puberty and commence its reproductive cycle.

Puberty

Puberty may be defined as the age at which the generative organs become functional and reproduction may occur but does not signify full or normal reproductive capacity (Roberts, 1986). In the female, puberty is characterised by the onset of oestrus and ovulation.

Puberty occurs at 5 to 7 months in does and 6 to 9 months in ewes (Jainudeen and Hafez, 1987). The Merino sheep and Angora goat are 18 to 20 months old at first oestrus. Early maturing breeds such as Pygmy goat reach puberty as early as 3 to 4 months. Puberty in tropical goats occur at the age of 249 to 293 days but most goats are bred after they reach 1 year old (Raja, 1977). The indigenous goat of Malaysia, Kambing kacang is first mated between 12 and 15 months (Devendra, 1983).

Oestrous Cycle

In all species of domestic animals that have reached puberty a new phase of the functional activity of the reproductive system is expressed by the onset of first oestrus and if the animal is not served this transient phase is followed by metoestrus, dioestrus and proestrus. The chain of the physiological events that begin at one oestrus period and end at the next is termed as oestrous cycle.

During the oestrous cycle of the goat, the changes in progesterone concentration in the peripheral plasma are similar to those previously reported in the ewe and cow (Thorburn and Schneider, 1972). The oestrous cycle is hormonally controlled by the interrelated actions of the gonadotrophic hormones secreted by the anterior pituitary and the gonadal hormones secreted by the ovary. FSH is responsible for follicular growth and maturation of the ovum. As the follicles grow, the quantity of oestrogen in the blood is high (26.9 pg/ml) (Bono et al., 1983). When oestrogen level increases, it suppresses the FSH level and stimulates the pituitary to liberate luteinising hormone (LH). LH causes preovulatory enlargement of the follicle, inducing ovulation and subsequently a corpus luteum. Progesterone level on day of oestrus is 0.2 ng/ml in the goat (Thorburn and Schneider, 1972). The concentration increased to a maximum of 4 ng/ml on day 10 of the 21 days cycle and decreased rapidly during the last 3 days of the cycle due to

regression of the corpus luteum which in turn stimulates the pituitary to liberate more FSH. Thus, a new cycle commences. However, during anaesthesia and surgery stress (Thorburn and Schneider, 1972), progesterone concentrations markedly increase (9 ng/ml).

Seasonality

Many wild species of bovidae are seasonal breeders with spring and summer bei
births. During the course of domestication, cattle were selected against seasonality, facilitating them to ovulate and conceive throughout the year. Sheep and goat in the temperate zone are seasonally polyoestrus and young are born in spring, the most favourable time of the year, whereas in the tropical zones, with less variation in day length, indigenous sheep and goats tend to breed throughout the year. 'Creole' meat goats maintained in good condition did not show marked seasonal variations in oestrus behaviour and ovarian activity (Chemineau, 1986) in the tropics. High environmental temperature and lack of feed may restrict sexual activity during some months of the year in the tropics, but shortly after the onset of the rainy season, sexual activity increases, probably due to the availability of feed and with decreasing day light (Hafez and Jainudeen, 1987).

Cycle Length

The length of the normal oestrous cycle in the ruminant ranges between 14 and 29 days. The average length of oestrous cycle is 21 days for cattle, water buffalo and the goat (Smith, 1978; BonDurant, 1981; Camp et al., 1983) and 17 days for the sheep (Foster, 1981). The average length of the cycle is 20 days for Kambing kacang (Devendra, 1983; Thangavelu and Mukherje, 1983), 20 days for the Indian Pashmina goats (Mazumder and Mazumder, 1983) and 21 days for the Angora goats (Ilgaz and Sernie, 1982). In the goat, active immunization against oxytocin causes a significant prolongation of the oestrous cycle, reflecting a lengthening of the functional life-span of the corpus luteum (Cooke and Homeida, 1985). In general, there is considerable variation in sheep and goat due to breed differences, stage of the breeding season and environmental stress.

Oestrus

Signs of Oestrus

In ruminants, signs of oestrus vary among species. The prominent signs of oestrus in cattle are bellowing, restlessness, swollen and hyperemic vulva, clear, stringy mucus discharge from the vagina, frequent urination and homosexual behaviour (Zartman et al., 1983).

Signs of oestrus are more conspicuous in does than in ewes. The buck or his odour will stimulate most nonpregnant does to show definite signs of oestrus. These signs include a rapid sideways or up-and-down tail wag which continues when hand pressure is applied to the doe's rump. The vulva may be swollen while milk production and appetite tend to decrease. Many does are more vocal than usual; they follow the buck or pace the perimeter of the enclosure searching for the buck (Smith, 1978; BonDurant, 1986). A doe may occasionally exhibit homosexual behavior (Smith, 1978). Thus, in the absence of a male, oestrus is difficult to detect in both the ewe and doe.

Duration

Duration of oestrus is influenced by breed, age, season and presence of the male. In general, duration of oestrus in the ewe and doe lasts 24 to 36 h and 24 to 48 h respectively (Jainudeen and Hafez, 1987). For the Kambing kacang oestrus lasts for 34 h (Devendra, 1983; Thangavelu and Mukherje, 1983) whereas for the Indian Pashmina and Angora goats, duration of oestrus lasts for 25 (Mazumder and Mazumder, 1983) and 35 h respectively (Ilgaz and Sernie, 1982).

Ovulation

Ruminants such as cattle, sheep and goats are spontaneous ovulators. Ovulation is controlled by gonadotrophic hormones: FSH and LH. The Graafian follicle is the major site for the

production of oestrogens. Twenty-four h prior to ovulation, LH released from the pituitary in a surge (ewe: 50-150 ng/ml) (Geshwind and Dewey, 1968; Pant et al., 1977) (goat: 30-50 ng/ml) (Bono et al., 1983), induces the final preparation of the follicle for ovulation. In goat, the LH preovulatory peak occurs 8 to 24 h after the onset of oestrus (Bono et al., 1983) but the interval from the onset of oestrus to the LH discharge in the ewe varies between and within breeds (Thimonier, 1979). In the Ile-de-France adult females, this interval is longer for ewe with 2 ovulations than with single ovulation and in highly prolific Romanov ewes (17.6 h) than in less prolific breeds (6 to 7 h) (Land et al., 1973). At ovulation in the goat, the level of LH in the blood reduces (0.5-3.0 ng/ml) rapidly but the FSH level begins to increase (11-17 ng/ml) (Bono et al., 1983). After the egg is shed, the ruptured follicle reduces in size and the granulosa cells become the lutein cells which are infiltrated with capillaries. This newly formed body is the corpus luteum and is responsible for progesterone production. At ovulation, the progesterone level in the blood is at basal level (0-0.8 ng/ml) but increases about 24 h after ovulation (1-4 ng/ml) (Bono et al., 1983).

Ovulation Time

Unlike cattle, both ewe and doe ovulate during the latter half of oestrus. The ewe and many breeds of goat normally ovulate about 24 to 27 h and 24 to 36 h respectively after the