UNIVERSITI PUTRA MALAYSIA

EFFECTS OF OESTRUS SYNCHRONIZATION PROTOCOLS AND ARTIFICIAL INSEMINATION TECHNIQUES ON CONCEPTION RATES OF BOER CROSSBRED DOES IN MALAYSIA

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By

NASSER SALEH OBAD HEZAM

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

August 2008

DEDICATED TO

MY FATHER, MY MOTHER, MY WIFE, MY CHILDREN, MY BROTHERS, MY SISTERS, MY FRIENDS, AND MY COUNTRY

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

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Chairman: Associate Professor Abd Wahid Haron

Faculty: Veterinary Medicine

A total of 83 Boer crossbred does weight between 14 - 44 kg were randomly selected and allocated into 2 groups. The first group of does (34) were divided into two subgroups: Group A (n = 21 does) and Group B (n = 13 does). All does in Group A (GA) were synchronized using CIDR containing 0.3 g progesterone for 17 days and injection of 50 µg $PGF_2\alpha$ and 250 IU of eCG at CIDR removal. Does in Group B (GB) were synchronized using CIDR containing 0.3 g progesterone for 17 days and injection of 50 µg $PGF_2\alpha$ without eCG at CIDR removal. All does in GA and GB were naturally mated using two bucks. Oestrus response, time and number of mating were recorded at 24-72h after CIDR removal. The does were observed twice daily at 7 am and 5 pm for two hours for three days. In the second group, 49 does were allocated into three subgroups G1 (n=20), G2 (n=14) and G3 (n= 15). All does were synchronized using CIDR for 17 days. At CIDR removal all does were injected intramuscularly with 50 µg $PGF_2\alpha$ and different doses of 100, 200 and 250 IU of eCG

for G1, G2 and G3 respectively. At 48-56 h after CIDR removal all does were inseminated with 0.25 ml frozen semen contains 25 $\times 10^6$ sperm/straw by different artificial insemination techniques using the transcervical (TC), intracervical (IC), intravagina (IV), or intrauterine (IU). Result of the study showed that oestrus responses were 100 % and 53.85 % for GA (CIDR with eCG) and GB (CIDR without eCG) respectively. There were significant difference ($p<0.05$) in oestrus responses between the two groups. Shortest oestrus responses were observed in GA, followed by GB. Conception rates for the groups GA and GB were 24% (5/21) and 38.5% (5/13) respectively. There were significant difference ($p<0.05$) in the conception rates of GA and GB. Conception rates using four AI techniques were 0% for G1 and G2. In G3 the conception rate were 40%, 25%, 0% and 0% following TC, IC, IV and IU AI techniques respectively. There were significant difference ($p<0.05$) in the conception rate among the three groups with different concentration of eCG, there were no significant difference in the conception rate among the different methods of AI used in all groups. For group G3 the conception rates by artificial insemination technique using TC is better than the IC, IV and IU techniques. This study suggested that the synchronization protocol of CIDR with eCG for 17 days were found better than CIDR without eCG. TC technique is preferred than IU using frozen semen in crossbred Boer does that were synchronized using CIDR for 17 days and injected with 250 IU of eCG and 50 µg PGF2α at CIDR removal.

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

KESAN PELBAGAI PROTOCOL PENSINKRONIAN ESTRUS DAN TEKNIK PERMANIAN BERADAS KE ATAS KADAR KONSEPSI KAMBING BETINA KACUKAN BOER DI MALAYSIA

oleh

Nasser Saleh Obad Hezam

Ogos 2008

Pengerusi: Prof Madya Dr Abd Wahid Haron
Fakulti: Perubatan Veterinar

ABSTRAK

Sejumlah lapan puluh tiga ekor kambing betina kacukan Boer berat antara 14-44 kg dipilih secara rawak dan diagihkan kepada dua kumpulan. Dalam kumpulan pertama, kambing dibahagikan kepada dua sub-kumpulan; GA (n=21) dan GB (n=13). Semua kambing disinkroni menggunakan CIDR yang mengandungi 0.3g progesterone selama 17 hari. Semasa mengeluarkan CIDR, kesemua kambing dalam GA disuntik secara intraotot dengan 50 µg $PGF_2\alpha$ dan 250 IU eCG sementara bagi GB pula diberikan suntikan 50 µg $PGF_2\alpha$ tanpa eCG. Semua kambing dalam dikahwinkan secara tabii menggunakan dua ekor pejantan. Reaksi estrus, masa dan bilangan kahwin direkodkan dalam 24-72 jam selepas CIDR dikeluarkan. Bagi reaksi estrus, kambing diperhati sebanyak dua kali sehari pada jam 7 pagi dan jam 5 petang selama dua jam.

Dalam ujikaji kedua pula, kambing dibahagikan kepada tiga kumpulan; G1 (n=20), G2 (n=14) dan G3 (n=15). Semua kambing disinkroni menggunakan CIDR yang

mengandungi 0.3 g progesterone selama 17 hari. Semasa mengeluarkan CIDR, kesemua kambing disuntik secara intraotot dengan 50µg $PGF_2\alpha$ dan pelbagai dos eCG diberikan sama ada 100, 200 and 250 IU masing-masing kepada G1, G2 dan G3. Di antara 48-56 jam selepas CIDR dikeluarkan, semua kambing di inseminasi dengan 0.25 ml semen beku mengandungi 25×10^6 sperma/straw menggunakan pelbagai teknik permanian beradas iaitu transervik (TC), intraservik (IC), intravagina (IV), atau intrauterus (IU). Keputusan menunjukkan bahawa bagi reaksi estrus, terdapat perbezaan bererti ($p<0.05$) di antara kumpulan di mana reaksi esturs yang singkat diperhatikan pada GA berbanding GB. Reaksi estrus bagi GA (CIDR + eCG) adalah 100% sementara bagi GB (CIDR tanpa eCG) pula adalah 53.85%. Reaksi estrus tersingkat diperhatikan pada GA dan diikuti oleh GB. Kadar kebuntingan bagi GA dan GB masing-masing adalah 5/21 (24%) dan 5/13 38.5%. Terdapat perbezaan bererti ($p<0.05$) pada kadar kebuntingan di antara GA dan GB. Keputusan juga menunjukkan kadar konsepsi menggunakan teknik AI adalah 0% untuk G1 dan G2. Kadar konsepsi dalam G3 pula adalah 40%, 25%, 0% and 0% masing-masing untuk teknik AI secara TC, IC, IV dan IU. Terdapat perbezaan bererti ($p<0.05$) di kalangan ketiga-tiga kumpulan dengan konsentrasi eCG yang berbeza tetapi tiada perbezaan bererti di kalangan kumpulan untuk teknik AI yang berbeza. Kadar konsepsi dalam G3 dengan menggunakan teknik TC adalah lebih baik daripada teknik IC, IV dand IU.

Kajian ini menunjukkan bahawa protocol pensinkronian estrus menggunakan CIDR + eCG untuk tempoh 17 hari didapati lebih baik berbanding CIDR tanpa eCG. Teknik AI secara TC adalah lebih baik berbanding IU dengan menggunakan semen beku pada

kambing kacukan Boer yang diseragamkan estrusnya menggunakan CIDR selama 17 hari dan disuntik dengan 250 IU eCG dan 50μg PGF2α semasa CIDR dikeluarkan.

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APPROVAL

I certify that an Examination Committee has met on date of viva to conduct the final Examination of Nasser Saleh Obad Hezam on his degree thesis entitled "Effects of oestrus synchronization protocols and artificial insemination techniques on conception rates of Boer crossbred goat in Malaysia" in accordance with University Pertanian Malaysia (Higher Degree) Act 1980 and University Pertanian Malaysia (Higher Degree) Regulations 1981. The Committee Recommends that the candidate be awarded the relevant degree. Members of the Examination Committee are as follows:

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

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DECLARATION

I hereby declare that the thesis is based on my original work except for quotations and citations which have been duly acknowledged. I also declare that it has not been previously or concurrently submitted for any other degree at UPM or other institutions.

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NASSER SALEH OBAD
Date: 5 June 2008

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LIST OF ABBREVIATIONS

S.E.M	Standard error of the mean
TAI	Timed artificial insemination
ART	Assisted Reproductive Technology
MPA	Medroxyprogesterone acetate
eCG	equine chorionic gonadotrophin
FGA	Fluorogestone acetate
PMSG	Pregnant mare serum gonadotrophine
CIDR-G	Controlled internal drug release device for goat
CIDR-S	Controlled internal drug release device for sheep
ES	Oestrus synchronization
CL	Corpus lueteum
PGF2α	Prostaglandin $F_{2\alpha}$ analogue
µg	Micro gram
IM	Intramuscular
hCG	human chorionic gonadotrophin
ET	Embryo transfer
IBHK	Institute Bioteknologi Haiwan Kebangsaan
TCAI	Transcervical Artificial Insemination
ICAI	Intracervical Artificial Insemination
IVAI	Intravagina Artificial Insemination
IUAI	Intrauterine Artificial Insemination
SPSS	Statistical Package for the Social Sciences
SEO	Synchronization of estrus and ovulation
MOET	Multiple ovulation and embryo transfer
LOPU	Laparoscopic ovum pick-up
AI	Artificial insemination
EE	Electro-ejaculator
AV	Artificial vagina
CASA	Computer assisted sperm analysis

CHAPTER 1

INTRODUCTION

The goat (*Capra hircus*) is one of the most important agricultural animals in the third world country, such as Yemen. Apart from supplying the important products, such as meat, milk, fibre and hides, the goat also provides fertilizers and serve as lawn mowers. Generally, goats can withstand harsh climates and thrive on poor forage. However, the goat, unlike cattle and sheep, has only received attention it deserves from scientists and breeders a few decades ago, although it is one of the oldest of the domesticated animal.

According to the Food and Agriculture Organization Production Year book (FAO, 2003) the world's goat population stands at 767,930,400 heads and China is the top goat meat producer in the world followed by India, Pakistan, Nigeria and Bangladesh. As the popularity of meat goat expands, there is increased interest in reliable technology to synchronize oestrus in goat. Goat meat producers can use this technique to complement other assisted reproductive technologies such as artificial insemination (AI) for reproductive management.

Oestrus synchronization permits kidding to occur at suitable times in order to take advantage of feed and labour, availabilities, markets, and increasing price trends. In the past, synchronization of oestrus focused only in cattle, sheep and dairy goats for optimal timing of milk production. However, with the upcoming interest in meat goat production, attempts have been made to use synchronization programmers of sheep and dairy goats in meat goats.

The various methods of oestrus synchronization that have and can be used include daylight hour's alteration and timed hormonal treatment combined with light alteration and buck effect. Research's have shown that timed hormonal treatment is the most convenient and successful method to synchronize oestrus in many meat goat production farms (Whitley and Saccksan, 2004). The introduction of breeds developed in other countries such as the Boer goats from South Africa and with increasing producer and consumer interests have increased the impetus for developing effective oestrus synchronization regimes and complemented with artificial insemination. As we are aware, current AI methods for goats depend on oestrus synchronization techniques that are adapted to the management system and practical conditions of the farm.

In Malaysia synchronization and AI in goats are hardly practised. The farmers in Malaysia depend mainly on the natural oestrous cycle of the indigenous goats and usually the herds are made up of a small number of goats. The proper implementation of natural mating and AI following oestrus synchronization can considerably increase performance of goats in a herd. Thus with this in mind, the main objective of this study was to determine and compare the effectiveness of AI techniques and natural mating on the conception rate of Boer crossbred goats following different oestrus synchronization protocols.

The specific objectives of the study were:

1. to compare the oestrus responses and conception rates following different synchronization protocols in natural mating does.

2. to determine the conception rate following different techniques of AI, with different doses of eCG, and

3. to compare the conception rate between AI and natural mating following different synchronization protocols.

There are several limitations in this study. These include:

1. Limited number of does used in this study is very much depending on its availability at the farm. As a result, there are some inconsistencies in the body weight of the does selected for the study.

2. The concentration of sperm in the frozen semen supplied by IBHK is 25 million /straw, which could be one of the reasons which have reduced the conception rates of animals in the farm.

3. Limited number of bucks available at the farm. Only two approximately health bucks were used.

CHAPTER 2

LITERATURE REVIEW

2.1 Goats

Goats are one of the oldest domesticated species. For thousands of years, goats have been reared for their milk, meat, hairs, and hides (Coffey, 2004). Goats' hides have been used to store water and wine for travelling. It has also been used to produce parchment, which was the most common material used for writing in Europe before the invention of the press (Coffey, 2004). Domestic goats are generally kept in herds that wandered on hills or other grazing areas but often tended by goat-herds who are frequently children.

The goat has proven to be the most adaptable of all the domesticated livestock and thus, can survive in a wide range of environments. However, when taken out of one environment and placed in another, it does not always portray its production potential. Angora goats imported into Sahelian West Africa performed very poorly due to the harsh environment (Wilson, 1992). Alpine and Saanen dairy goats imported into India performed only somewhat better than local breeds under a stall management system (Devendra and Burns, 1983).

Males and females should be separated before 16 weeks of age because it is possible for fertile matings to occur at ages between 4 and 6 months (Gregor, 2007). The recommended breeding age is about seven months but mating at this age is not

generally recommended as it can be costly since it is likely to reduce the life-time of kid production of the doe (Zeder and Hesse, 1999).

Generally, the kidding performance of does less than 25 kg body weight is very poor during the first year, and that life-time production is reduced by 10 to 40% and up to 30% of does mated at an early age abort during the later part of pregnancy (Gregor, 2007). Therefore for weaner does that are used for mating, their body weight should 25 kg or more and they must be fed well during pregnancy and lactation (Zeder and Hesse, 1999).

The most common method of induction oestrus involves the use of sponges impregnated with progesterone or CIDR or a synthetic version of this. Goats are usually mated by natural matings and for pedigree mating a single female is brought to a male (Zeder and Hesse, 1999).

2.2 Methods of breeding

Two methods of breeding are practised in goats: natural mating and artificial insemination (AI). In natural breeding, a doe in heat is bred to a buck (Ax et al., 2000; Pugh, 2002). Artificial insemination involves collection of semen from a buck and transfer of the semen to the reproductive tract of the doe (John and Smith, 1996).

2.2.1 Natural Breeding

Natural breeding is more commonly used in meat and fibre sheep or goat production systems, and this method is most commonly used by purebred breeders. In meat

production system, productivity is largely a function of the number of offspring born, weaned and the frequency with which they are produced (Pugh, 2002). Females are usually bred in the fall for spring kidding. Bucks should be kept separate from the does until they are to be used for breeding (Ax et al., 2000; Pugh, 2002).

After establishing a mating time, the producer should leave the bucks with the does for 32 days (Pugh, 2002). This ensures that all kids are born within a month of each other, reducing the amount of supervision required at kidding time. The male to female mating ratio depends on the age and season condition of the male, the size of the mating area, and whether one or more bucks are to be used. Meat goat production systems should have 1 buck per 30 does. A buck may breed 50 to 200 does in a single breeding season, but 3 to 4 bucks should be put with 100 does (Ax et al., 2000; Pugh, 2002).

A marking harness should be used on the males to mark and identify females that have been mounted and bred. In commercial flocks it is recommended that buck be changed at least every 2 years to prevent inbreeding (Pugh, 2002). Bucks of high libido and with good semen quality can be used in a staggered breeding program in which synchronized does exhibiting oestrus at the same time are placed with those males for breeding (Ax et al., 2000; Pugh, 2002).

Advantages and disadvantages of natural breeding; goats have been breeding naturally for centuries and an owner of a doe must decide which buck to use, and the chosen buck takes care of the rest (Pazzani, 2008). However, if do not want to breed does with your own buck, other bucks from different owners can be used with a fee

and in some areas, this fee varies depending on the quality of the buck (John and Smith, 1996; Cassese and Smith, 2007 and Pazzani, 2008).

A doe will be on heat for 12 to 36 hours, and after breeding, the semen can live for about 24 hours in the reproductive tract of the doe. Therefore, it is not essential to breed at the exactly time, because the breeding process can be repeated for the next few days (John and Smith, 1996; Cassese and Smith, 2007 and Pazzani, 2008).

On the other hand, there are some disadvantages of natural breeding. Stronger fences are required for the bucks and usually they are housed away from does. During breeding seasons, bucks have some unusual behavior such as urinating on their beards (John and Smith, 1996; Cassese and Smith, 2007). The cost to purchase bucks is normally higher than does and to maintain the bucks is more expensive because the bucks eat more than the does and also destroy the infrastructure more frequently (John and Smith, 1996; Cassese and Smith, 2007 and Pazzani, 2008).

2.2.2 Artificial Insemination

Artificial insemination (AI) is the assisted reproductive biotechnology applied to improve reproduction and genetics of farm animals (Ax et al., 2000; Baldassarre and Karatzas, 2004). It had an enormous impact worldwide in many species, particularly in dairy cattle. The acceptance of AI technology worldwide provided the impetus for developing other technologies, such as cryopreservation and sexing of sperm, oestrous cycle regulation, and embryo harvesting, freezing, culture and transfer, and cloning. Many of the pioneers helped to develop a new generation of reproductive

physiologists and biotechnologists. Use of AI in animals is an accidental human invention and much of the development of AI occurred before the 1980s when electronic networks became available. The development of AI becomes more important when it is used in animal biotechnology to improve semen collection, evaluation, preservation, and insemination. Detection of oestrus and control of the oestrous cycle in the female was also important (Foote, 2002).

The advantage of AI is to maximize the use of outstanding sires' bucks and eliminate the need of bucks on the farm and relatively cut down the cost and decreased potential for venereally disease transmission and also improve the herd management (John and Smith, 1996; Ax et al., 2000; Pugh, 2002; Cassese and Smith, 2007 and Pazzani, 2008). The disadvantage of AI includes the expensive cost of AI equipment and liquid nitrogen and increased labour for oestrus detection and insemination. There is a lack of standardization procedures for packing and quality control for goat semen and also suitable sire to proof the production trails and this will lead to less desirable traits (John and Smith, 1996; Pugh, 2002; Cassese and Smith, 2007 and Pazzani, 2008).

2.2.2.1 Artificial Insemination (AI) in Goats

The early development of AI in goat on a major scale began in Russia (Maule, 1962 and Milovanov, 1964) where the collective farms provided an ideal arrangement for establishing AI programs. Then on, Artificial insemination spread to central Europe and was widely applied commercially in France and Brazil (Maule, 1962 and Foote, 1999). China also has extensive sheep AI programs (Foote, 2002).

Artificial insemination may be regarded as a first generation assistant's reproduction technology (ART) and it is the one that has made the greatest contribution to genetic improvement programs, mainly due to well-established methods for identifying males with the highest genetic merit. Artificial insemination is a process by which sperm is collected from the male, processed, stored fresh or frozen and artificially introduced into the female reproductive tract for the purpose of conception (Ax et al., 2000; Baldassarre and Karatzas, 2004).

Artificial insemination has become one of the most important techniques ever devised for the genetic improvement of farm animals. It has been most widely used for breeding and has made males of high genetic merit available to all. Female can be inseminated with either fresh semen or with commercially available frozen semen (Baldassarre and Karatzas, 2004).

The success of an AI program depends on many factors: whether fresh or frozen semen is used, number of times insemination during oestrus, time and method of insemination, and location (vagina, cervix, trancervical or uterus) of insemination, quality and quantity of semen inseminated (number of live sperm cells), semen (fresh or frozen) handling for AI, and management of the animals to be inseminated (Ax et al., 2000; Pugh, 2002).

Goat artificial insemination allows individual bucks to be exploited widely and so is a potentially useful tool for rapid genetic improvement of especially fibre goats. Artificial insemination may best be adopted under extensive grazing conditions using