# CHAPTER 1

# 1.0 INTRODUCTION

## 1.1 BACKGROUND

Goat farming in large scale is a potential livestock industry in Malaysia. In 2010, it was estimated that there were 545,682 goats in Malaysia with ex-farm value of RM68 million. The total production of local mutton was 2,386.5 metric tonnes, just about 10% of the total consumption of 22,549 metric tonnes per year (Department of Veterinary Services, 2011). The population of goats in Malaysia mainly consists of various crossbred goats and local Kambing Katjang. The crossbreds are the results of indigenous Kambing Katjang being bred with one or more of the imported breeds like Boer, Jamnapari, Saanen, Alpine, Toggenburg and Anglo-Nubian. The crossbred goats are categorised into various grades and genetically heterogenous animals. Large proportions of these crossbred goats have undergone generations of interbreeding and they are classified with the Kambing Katjang as local goats. The mean adult body weight of Kambing Katjang is 20-25 kg, meanwhile the local crossbred goats are larger with mean adult male and female body weight of 36 kg and 30 kg, respectively ((Department of Veterinary Services, 1988).

It is challenging for the industry to achieve 100% self-sufficiency of mutton consumption due to lack of breeders in this country, among other factors. Goats genetic improvement practice using conventional methods of natural mating and artificial breeding were shown to relatively slow progress and utilise only superior genetic material from male side. Innovative development in assisted reproductive technologies (ART) such as multiple ovulation and embryo transfer (MOET), embryo manipulating and cryopreservation could accelerate the production of superior genes which offer hope

for model and designed animals to be propagated more rapidly to fit the current market and environment requirements.

In the past few decades, there has been tremendous progress in the development of the ART for the purpose of genetic improvement in farm animals. As for artificial insemination (AI), embryo transfer (ET) has considered as one of the most encouraging development in the reproduction of farm animals. The procedure of MOET has the potential to accelerate genetic improvement through the contribution of superior genetic merit from both the male and female sides to the population gene pool. The activities of MOET for the purpose of genetic improvement normally involved the procedures of superovulation of genetic superior donor, followed by recovery of embryos and transfer to appropriately synchronised recipients. Beside superovulation procedure and *in vivo* embryo recovery, Tibary *et al.* 2005 indicated that improvements in oocycte collection and maturation technique for the *in vitro* embryo production (IVP) in small ruminants through *in vitro* maturation (IVM), *in vitro* fertilisation (IVF), intracytoplasmic sperm injection (ICSI), or nuclear transfer (NT), provided an excellent source of embryo production for ET.

There are many advantages of using MOET: It allows a large number of progenies from genetically superior females to be born by shortening generation interval, easier introducing of exotic breeds into a country through importation of embryos, conserving endangered species, enhancing progeny testing, reducing the risk of introducing exotic diseases, minimising cost and eliminating transportation stress in animals, obtaining twins and multiples from each pregnancy, building separate flocks for meat and milk breeds from a few superior animals and utilising genetically inferior females as foster mothers for embryos (Ishwar and Menon, 1996). Coupled with DNA technologies such as sex selection, gene transplant and cloning, various progeny of livestock genetic alterations are possible to be produced (Gootwine *et al.,* 1997).

MOET is an appropriate tool for accelerate superior genetic progress in which superovulation is one of determinant factors because of variability of the superovulatory response in goats. At present, there is no optimal superovulatory protocol established for goats. Therefore it is justified to carry out superovulation programme with consistent ovulation response (Nowshari *et al*., 1995). The aims of superovulation were to induce a high number of ovulation subsequent fertilisation and to ensure a favourable physiological environment in the reproductive tract for embryo development in the donor animal (Kafi and McGowan, 1997). Many factors could affect the variation in ovarian response of superovulated goat. These include factors related to the physiological status of the animal (intrinsic factors) such as age, genetic differences and ovarian status at the time of treatment. Extrinsic factors including season, nutrition and hormone preparations (Baril *et al.*, 2000; Gonzalez-Bulnes *et al*., 2003a).

The superovulation treatment is usually given toward the end of the synchronisation treatment. Gonadotrophin is normally used to stimulate the ovaries and increase the number of ovulation (Thibier and Guerin, 2000). Exogenous gonadotrophins have been widely employed as a means of superovulation in livestock embryo transfer (Jabbour and Evan., 1991a,b). The main factor attributed to variation and unpredictability in the ovulatory response obtained by superovulation is the type and preparation of the exogenous gonadotrophins employed (Picazo *et al*., 1996; Cognie, 1999; Gonzalez-Bulnes *et al*., 2003c). Currently, the most common exogenous gonadotrophins preparation available for goat superovulation are FSH and eCG. In caprine MOET programmes, FSH preparation are mostly utilised (Jabbour and Evan, 1991b; Espinosa-Marquez *et al*., 2004). Due to the short half-life (5 hours) of the molecule (Demoustier *et al*., 1988), FSH is administered every 12 hours for a period of 3 to 4 days. FSH with appropriate admixture of luteinising hormone (LH) gives acceptable superovulatory results.

Beside the type and the preparation of the exogenous gonadotrophins used in the superovulation treatment, the total concentration of FSH and the distribution pattern of the FSH doses (i.e. equal vs. decreasing) are also contributing factors to the variation of ovarian response obtained (Thibier and Guerin, 2000). There is a general consensus that decreasing doses give higher responses in term of numbers of ovulations than a equal dose level (Baril *et al*., 2000; Gonzalez-Bulnes *et al*., 2003a). There have been several attempts to devise more simple and less labour intensive treatment regimes without compromising the ovarian response and embryo production. Compatible ovarian response had been reported by substituting the last three of six FSH-injections by a single dose of 200 IU eCG (Pintado *et al*., 1998; Batt *et al.*, 1993; Baldassarre *et al*., 2002 and Baldassarre *et al.,* 2003). Meanwhile, in ewe, a single injection of high dose of FSH given 12 h before withdrawal of progesterone increase estrous response, ovulation rate, conception rate, but had no overall effect on pregnancy percentage rate (Knights *et al*., 2003). The simplicity of the "one shot"-treatment is appealing.

Previously, the main variable factor in ovarian response of FSH-superovulated goat was attributed to the content of FSH:LH ratio in the gonadotrophin (Nowshari *et al*., 1995). A high LH content in the FSH preparation generally leads to incidence of premature ovulation of the large follicles stimulated. This event will trigger the production of progesterone and altering the progesterone and oestrogen circulating concentration ratio and eventually disturbs the maturation process of ova with result in production of poor quality embryos (Baril *et al.,* 1996; Nowshari *et al*., 1995; Pintado *et al*., 1998; Holtz, 2005, Saharrea *et al.,* 1998). Low LH concentration in more purified FSH preparations such as Ovagen (oFSH) and Folltropin (pFSH) are currently available and are used to overcome the incidence of premature ovulation. However, these purified FSH preparations lead to other incidences of abnormal ovulations and unovulated follicles (Pintado *et al*., 1998; Gonzalez-Bulnes *et al*., 2003a). This phenomenon,

however, was reduced by the supplementation of LH, hCG and GnRH to help the ovulation process (Saharrea *et al*., 1998).

Equine chorionic gonadotrophin (eCG) has also been widely utilised for superovulation in goats with limited success compared to FSH (Armstrong *et al*., 1983; Mahmood *et al*., 1991; Holtz, 1996; Saharrea *et al*., 1998 and Cognie, 1999). This might be associated with long half-life of 10-15hours (Holtz, 2005). Long half-life has been shown to cause over-stimulation of follicular growth, led to the production of large unovulatory follicles following superovulation or cystic follicles that altering estrogen and endogenous $PGF_{2\alpha}$ levels then leads to high incidences of premature luteal regression. These event result a decline in progesterone concentration before embryo collection, leading to a loss in embryos due to embryo toxicity (Stubbing *et al*., 1986; Saharrea *et al*., 1998, Buford *et al*., 1996; Costine *et al*., 2001; Espinosa-Marquez *et al*., 2004).

The variation in response from the superovulation is also induced by inadequate progesterone levels to block the LH pulses towards the end of the progestagen treatment during oestrus synchronisation period. Inadequate progesterone levels during this period, consequently extending the life span of a dominant follicle and leads to variability on the onset of oestrus and ovulation (de Castro *et al*., 1999; Kim *et al*., 2003). Extending the life span of a dominant follicle will increase the oestrogen secretion and alter the competence of the newly stimulated oocytes through superovulation, thus the production poor quality of oocytes leading to increased embryonic mortality. The critical part of variability in the onset of oestrus and ovulation is being the miss-timing of ovulation, which leads to a high recovery of unfertilised ova, resulting in poor embryo recovery, especially following fixed-time AI (Moore and Eppleston, 1979; Evans and Armstrong, 1984; Brebion *et al*., 1992). Impeded sperm transport and abnormal maturation of oocytes are also the main factors contributing to

the fertilisation failure following superovulation in goat (Kumar *et al*., 1990, 1991). Impeded sperm transport, generally associated with natural and cervical inseminations. Intra-uterine insemination can, therefore, improve the fertilisation rate (Armstrong and Evans, 1983; Evans and Armstrong, 1984; Hawk, 1988; Kafi McGowan, 1997; Cognie, Baril, Poulin and Mermillod, 2003).

Repeated superovulation for a period of time also can reduce the ovulation rate, embryo yield and viability of the embryos recovered. It has been found that successive superovulation procedures lead to the formation of gonadotrophin antibodies (Nuti *et al*., 1987; Beckers *et al*., 1990; Baril *et al*., 1996; Holtz, 2005). Repeated surgical embryo collection in goats on the other hand, can leads to the formation of post-operative adhesions. The formation of the adhesions limits the number of times that a donor can be flushed to 2 to 3 times (McKelvey *et al*., 1985; Ishwar and Menon, 1996; Pereira *et al*., 1998; Suyadi *et al*., 2000). Nonsurgical methods of embryo collection in goats, laparoscopy and transcervical passage of the catheter through dilation of the cervix with $PGF_{2\alpha}$ or oestrogen could also be considered to overcome this problem (McKelvey *et al*., 1985; Pereira *et al*., 1998; Wulster-Radcliffe *et al*., 1999).

The state of the ovary at the onset of a superovulation is a major factor responsible for the variation in response to superovulation (Cognie *et al*., 2003; Gonzalez-Bulnes *et al*., 2003b, 2004a; Holtz, 2005). Therefore, the creation of an environment where there is a high number of gonadotrophin-responsive follicles (2-6 mm in size), no dominant follicle and the presence of a corpus luteum at the onset of a superovulatory treatment is essential. This condition can be achieved by the synchronisation of the ovarian follicular emergence through follicular ablation, avoiding the development of a dominant follicle by lowering LH concentration or by starting superovulation soon after ovulation at follicular wave emergence (Diskin *et al*., 2002; Gonzalez-Bulnes *et al*., 2002; Rubianes *et al*., 2003a;).

Intrinsic factors such as breed, season and age also contribute a big impact on the ovarian response to superovulation. The number of corpora lutea, recovered and transferable embryos differ with breeds (Bindon *et al*., 1986; Nuti *et al*., 1987; Baril *et al*., 1989; Dufour *et al*., 2000). In goats, more unovulatory large follicles have been observed during seasonal anoestrus than during the natural breeding season (Pendleton *et al*., 1986; Walker *et al*., 1989; Sebastian-Lopez *et al*., 1990; Mitchell *et al*., 2002). The effect of age of the doe in MOET programmes demonstrates some differences between ages. There is general agreement that juveniles can be superovulated and the follicles are sensitive enough to gonadotrophin stimulation (Donaldson, 1984; Driancourt *et al*., 1990; Rangel-Santos *et al*., 1991; Hasler, 1992; Kuhholzer and Brem, 1999). However, a poorer response to superovulation in terms of lower ovulation and fertilisation rates as well as lower embryo recovery and survival rates have been recorded in young females compared to adult females (Quirke and Hanrahan, 1977; Rangel-Santos *et al*., 1991; Driancourt and Avdi, 1993). The last stage of MOET, i.e. embryo transfer, requires the synchrony of oestrus between the donors and the recipients (Moore, 1974; Moore and Eppleston, 1979; Ishwar and Menon, 1996; Oppenheim *et al*., 2000; Holtz, 2005). Generally, two morulae or blastocyst are surgically transferred to the oviduct or uterine horn ipsilateral to the corpus luteum of the recipient with at least one normal corpus luteum being present on days 6 to 7 following oestrus (Traldi *et al*., 1999; El-Gayer and Holtz, 2001; Guignot *et al*., 2006).

## 1.2 JUSTIFICATION

The importance of this research are:

i. To provide a better understanding of the goat MOET programmes under local setting and to evaluate various factors affecting efficiencies of superovulatory response and kidding rate such as total dosage and pattern of administration in oFSH superovulation protocols related to breed and body weight; stages of embryo development and embryo quality; and the sources of embryo transferred.

ii. To elucidate some information on the intrinsic and extrinsic factors on MOET performance in goats in an attempt to make it more practical and economical in Malaysia.

iii. To provide reliable guidelines for goat breeders interested in MOET programme.

iv. To provide an option for goat breeders to breed and preserve their valuable goat genetic materials.

## 1.3 SCOPE OF THE STUDIES

This study focused on three different oFSH superovulation protocols for the donors and one synchronisation method for both donors and recipients. Fertilised *in vivo* embryos derived from natural breeding using bucks were transferred into synchronised recipients. *In vivo* embryos were surgically flushed on day 3 or 7 after CIDR withdrawal to produce different stages of embryos. Colleted viable embryos were then transferred in to oviduct or uterine of recipients via surgical method. The parameters measured numbers of donor does responded to oFSH superovulation protocol (more than 2 CL); number of ovulatory (CL) and anovulatory follicles (AF); total number of recovered structures (embryos plus ova); and total number of viable embryos for transfer. Number of embryos transferred; source and stages of embryo development; CL, number of pregnancy on day 60 and; number of kids born were also recorded.

## 1.4 OBJECTIVES

The general objective of this study was an attempt to develop an MOET programme in goats under Malaysia environment. Six specific objectives of this study were:

a) To assess the effects of dosages and pattern of oFSH administration on ovarian response in superovulated Malaysian goat.
b) To determine the effects of breed on ovarian response in superovulated Malaysian goat.
c) To determine the effects of body weight on ovarian response in superovulated Malaysian goat.
d) To assess the stage of development and quality of embryos retrieved on days 3 and 7 after CIDR withdrawal in superovulated-Malaysian goat.
e) To compare the efficiency of embryos transfer from ICSI and *in vivo* embryos.
f) To produce kids from the MOET procedure.