PRODUCTION OF GOAT OFFSPRING FROM *IN VIVO*-DERIVED EMBRYOS THROUGH EMBRYO TRANSFER TECHNIQUE

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ABSTRACT

Goat emAbryo transfer is an important tool to improve the genetics and to increase rapidly the number of economically superior goat for the industry. The objective of this study was to develop a suitable protocol for the collection and transfer of goat embryos as well as subsequently to produce goat kids through embryo transfer. Two different hormones (PMSG, FSH-V), two different localities (Kuala Lumpur, Malaysia and Kunming, China) and the embryo recovery cycle were designed to evaluate the ovarian responses and embryo transfer performance. For donor goats, the treatment was 14 or 17 days CIDR and before CIDR withdrawal, 1200 IU PMSG or 160 mg FSH-V were injected before CIDR withdrawal, and then followed by checking oestrus and natural mating. After day-7 CIDR withdrawal, uterine horn was flushed surgically to collect embryos. For recipient goats, similar CIDR administration was given and upon CIDR withdrawal, 300 IU PMSG was injected. On the same day, the collected embryos were transferred into the uterus of the recipient, in which it was confirmed by appearance of 1 to 2 corpus luteum on the ovaries. A total of 460 embryos were obtained from 122 donor does, out of which 174 embryos were transferred to 117 recipient does, resulting in the delivery of 73 kids in Kunming and 3 kids in Kuala Lumpur. Both donor and recipient does synchronised with 17 days of CIDR administration showed a higher ovulation rate compared with 14 days of CIDR treatment with the values of 90 and 67%, respectively. The ovulation and unfertilised oocytes plus embryos recovery rates were higher in the FSH-V (100 and 44%, respectively) than those of PMSG treated does (95 and 34%, respectively). The FSH-V treatment gave better results than PMSG treatment in both fertilisation rate (43.55% vs. 34.08%, respectively) and number of embryos

obtained (4.38±0.85 vs. 0.63±0.23, respectively). The ovarian responses and embryo transfer performances after FSH-V treatment were better in Kunming than in Kuala Lumpur (unfertilised oocytes plus embryos: 9.47±0.64 vs. 5.06±0.90; number of embryos obtained: 8.13±0.69 vs. 4.38±0.85; the pregnancy rate: 65.69 vs. 11.11%; kidding rate: 71.57 vs.0%, respectively). The total number of embryos recovered after superovulation of first (116 embryos) and second (80 embryos) flushing cycles were higher than third (4 embryos) flushing cycle. It is concluded that superovulation using specific hormones protocol and localities as well as the superovulation cycles are important factors for consideration in embryo transfer programme of goats.

Abstrak

Pemindahan embrio kambing adalah suatu kaedah penting untuk menambah baik genetik dan meningkatkan dengan cepat bilangan penghasilan kambing yang bermutu dari segi ekonomi untuk industry. Tujuan kajian ini adalah untuk menghasilkan satu protokol yang sesuai untuk pungutan dan pemindahan embrio kambing serta kemudiannya penghasilan anak kambing melalui pemindahan embrio. Dua hormon yang berlainan (PMSG, FSH-V), dua lokasi (Malaysia, China) dan kitar pungutan embrio direkabentuk untuk menilai respons ovari dan prestasi pemindahan embrio. Bagi kambing betina penderma, perlakuannya adalah 14 hari atau 17 hari CIDR dan sebelum mengeluarkan CIDR, 1200 IU PMSG atau 160 mg FSH-V telah disuntik serta diikuti dengan pemeriksaan estrus dan pengawanan semulajadi. Selepas hari ke-7, CIDR dikeluarkan, tanduk uteri dipam keluar secara surgikal untuk pungutan embrio. Bagi kambing penerima, pemasukan CIDR yang sama telah dilakukan dan sebelum CIDR dikeluarkan, 300 IU PMSG telah disuntik. Pada hari yang sama, embrio yang dipungut telah dipindah ke dalam uterus penerima di mana ia dikenalpasti dengan kehadiran 1 hingga 2 korpus lutea pada ovari. Sejumlah 460 embrio telah diperolehi daripada 122 kambing betina penderma, dimana 174 embrio daripadanya telah dipindahkan ke dalam 117 kambing betina penerima yang kemudiannya melahirkan 73 anak kambing di China dan 3 anak kambing di Malaysia. Kedua-dua kambing betina penderma dan penerima yang disinkronikan dengan pemasukan CIDR selama 17 hari menunjukan kadar ovulasi yang lebih tinggi berbanding dengan pemasukan CIDR selama 14 hari dengan nilai sebanyak 90 and 67%, masing-masing. Kadar ovulasi and kadar pungutan oosit yang tidak tersenyawa bersama embrio adalah lebih tinggi pada kambing betina yang diperlakukan dengan perlakuan

FSH-V (100 dan 44%, masing-masing) berbanding dengan perlakuan PMSG (95 dan 34%, masing-masing). Perlakuan FSH-V memberi keputusan yang lebih baik berbanding perlakuan PMSG dalam kedua-dua kadar persenyawaan (43.55% lawan 34.08%, masing-masing) dan bilangan embrio yang diperolehi (4.38±0.85 lawan 0.63±0.23, masing-masing). Respons ovari dan prestasi pemindahan embrio selepas perlakuan FSH-V adalah lebih baik di China berbanding di Malaysia (Oosit tak tersenyawa bersama embrio: 9.47±0.64 lwn 5.06±0.90; bilangan embrio diperolehi: 8.13±0.69 lwn 4.38±0.85; kadar bunting: 65.69 lwn 11.11%; kadar kelahiran: 71.57 lwn 0%, masing-masing). Jumlah embrio terkumpul selepas superovulasi dipam keluar bagi kitar pertama (116 embrio) dan kedua (80 embrio) adalah lebih tinggi berbanding kitar pam keluar ketiga (4 embrio). Kesimpulannya, superovulasi dengan menggunakan protokol hormon yang spesifik dan lokasi serta bilangan kitar superovulasi adalah faktor penting yang perlu diambil kira dalam program pemindahan embrio kambing.

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LIST OF ABBREVIATIONS

ABEL	animal biotechnology embryo laboratory
ART	assisted reproduction technologies
AI	artificial insemination
CL	corpus luteum
CIDR	controlled internal drug release device
DPBS	dulbecco phosphate-buffered saline
ET	embryo transfer
ES	oestrus synchronisation
FSH	follicle stimulating hormone
HAP	horse anterior pituitary extract
hCG	human chorionic gonadotrophin
hMG	human menopausal gonadotrophin
ICSI	intracytoplasmic sperm injection
IGF	insulin-like growth factors
IVC	*in-vitro* culture
IVF	*in-vitro* fertilization
LH	luteinising hormone
MOET	multiple ovulation and embryo transfer
NIH-FSH-P1	National Institute of Health-Follicles Stimulating Hormone-P1
PGF2α	prostaglandin F2α
PMSG	pregnant mare serum gonadotrophin
SEO	synchronisation of oestrus and ovulation

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Chapter 1

1.0 INTRODUCTION

Chapter 1

2.0 INTRODUCTION

1.1 GOAT BACKGROUND

The domestic goat (*Capra hircus*) is a subspecies of goat domesticated from the wild goat of Southwest Asia and Eastern Europe. The goat is a member of the Bovidae family and is closely related to the sheep as both are in the goat-antelope subfamily Caprinae. There are over 300 distinct breeds of goat (Hirst, 2008). Goats are one of the oldest domesticated species. Goats have been used for their milk, meat, hair, and skins over much of the world (Coffey *et al.*, 2004). The goat was one of the first animals to be domesticated by humans, about 9,000 years ago. Today, there are some 200 different breeds of goats that produce a variety of products, including milk, meat and fibre (mohair and cashmere). Worldwide, goat meat production is higher than meat production from cattle or pigs (Holcomb, 1994). Goats can survive in a variety of climatic conditions. It can even be found in very hot tropical rain forest as well as the arid desert region around the world. Therefore, goat can be considered as the most popular livestock animals in the world (Sahlu and Gvestsch, 2005).

Goat plays a significant role in poverty alleviation, especially in the developing countries. In Malaysia, the population of the goats is still relatively low which approaches 470,000 heads in 2008, considering the high goat meat requirement in the country (Department of Veterinary Services Malaysia, Agriculture Statistical Handbook, 2008). Most of the farmers who are poor small-holders, each posses small number of goats (2 to 10 goats) which supplements other sources of income for their families. According to the

current report in Malaysia, most of the frozen mutton (90% of goat meat requirement) is imported from New Zealand, Australia and Brazil.

The overall goat production can be increased by the substitution of genetically superior animals for those of low genetic merit. At present, reproductive biotechnologies are gaining popularity by integrating them animal in production system. Such technologies include artificial insemination (AI), oestrus synchronisation, superovulation, *in-vitro* fertilisation (IVF), intracytoplasmic sperm injection (ICSI), embryo cryopreservation, sperm cryopreservation, gender selection, pregnancy diagnosis by using ultrasound scanning, embryo transfer (ET), nuclear transfer and gene transfer.

1.2 EMBRYO TRANSFER BACKGROUND

Walter Heape (1891) was the first scientist to produce live young rabbits by embryo transfer. The first successful ET in goats was reported by Warwick *et al*., (1934), in which subsequently ET in small ruminants was popularly studied in Australia (Moore and Eppleston, 1976), New Zealand (Tervit *et al*., 1984), France (Chemineau *et al*., 1986) and India (Goel and Agrawal, 1990). ET is a systematic programme which consists of management of donor and recipient animals, synchronisation of oestrus in donors and recipients, superovulation of donors, breeding (natural mating or artificial insemination), embryo collection and transfer of embryos.

Synchronisation of oestrus and ovulation (SEO) is a key component of all the assisted reproductive technology (ART) protocols and has a major influence on the overall efficiencies of this programme. Since goats are seasonal breeders for the temperate breeds, therefore, SEO is the most widespread method of synchronisation used

in goats. The progesterone/progestagen treatment may be delivered by means of an intravaginal sponge (Ungerfeld and Rubianes, 1999), a CIDR or a subcutaneous implant. But the use of progestagen sponges in "long protocol" treatments (18-21 days) has been shown to result in lower fertility rates, possibly due to poor semen transport (Rubianes and Menchaca, 2003) proposed the use of a "short-priming protocol" in which goats are treated with progestagen for 5 days and 200-300 IU of eCG is administered at sponge/CIDR removal. Using their proposed "short-priming protocol" and eCG, a 68% pregnancy rate was achieved following fixed-time AI (54 hours from sponge removal) with fresh semen.

1.2.1 Multiple Ovulation and Embryo Transfer (MOET)

Superovulation is considered to yield a larger number of transferable embryos. An average of 6 to 8 transferable embryos per donor can be produced in a successful goat MOET programme (Baril *et al*., 1993a; Cognie *et al*., 2003). With MOET, there is an increase in the reproductive rate of female, especial in sheep (2.0 to 3.4) and cattle (1.5 to 2.0) (Smith, 1976).

At present, several strategies have been suggested for increasing the number of small recruitable ovarian follicles at the time of FSH treatment, while avoiding the presence of large (dominant) follicles. Some of these strategies include the use of GnRH agonist/ antagonists and the administration of FSH shortly after an induced oestrus/ovulation (Baldassarre and Karatzas, 2004).

The transfer of goat embryos to surrogate mothers is most commonly accomplished by surgical means. Recipients should be rejected if no corpus luteum is

present or pathologic condition of the reproductive tract is noted. Traditionally, recovery of embryos from the oviduct should be transferred to the oviduct. For oviduct transfers, the catheter containing the embryos was introduced 2 to 3 cm inside the oviduct and the medium containing embryos was deposited. Conversely, recovery of embryos from the uterus should be transferred to the uterus. Only the tip of the uterine horn ipsilateral to the ovary displaying an ovulation site needs to be exteriorised. For uterine transfer, a small zone of the uterine horn was exposed and perforated with a punch and the catheter with embryos was introduced in cervical direction and embryos were disposed in the uterine lumen.

1.3 JUSTIFICATION

Embryo transfer is a powerful tool for multiplication of superior animals. The application of ART enables the rate of genetic progress to be increased. Some of these techniques accelerate progressively by increasing the selection differential. The ART allows animals of high genetic merit to produce more offspring than would be possible by natural breeding. For example, in natural course, a single female doe will produce 1 to 3 progenies per year. Using superovulation and embryo transfer technologies, it is feasible to collect around 30 to 50 embryos from one female per year. Moreover, in combination with hormonal synchronisation of oestrus and superovulation, these techniques allow the production of goat offspring and milk in times of the year that are not the natural breeding period of seasonally reproductive species such as the goat (Leboeuf *et al*., 1998). The ET techniques are also able to increase the selection differential by shortening the generation interval (Baldassarre and Karatzas, 2004).

1.4 STATEMENT OF PROBLEMS

Major questions that not yet to be answered with regards to be answered with regards to embryo transfer in goat includes:

a) What is the suitable doe's age for embryo transfer?

b) Are there any effects of season, climate, longitude and latitude and temperature for embryos transfer in goat?

c) Which procedure is better for fertilisation, natural mating or artificial insemination?

d) When is the best time to do embryo flushing?

e) Which method is better for embryo flushing， oviduct flushing or uterine horn flushing?

f) How many embryos can be transferred into recipient does?

g) Which stage of embryos given better survival in recipient?

h) How many times can repeat the superovulation technique in same valuable donor?

i) How to solve the postsurgical complication problem?

j) What is the optimal dose of hormone for superovulation in goat?

k) How to improve the pregnancy rate after embryo transfer?

l) How to improve the survival offspring rate after embryo transfer?

1.5 OBJECTIVES

The general objectives of the present study were to obtain the embryos from PMSG-stimulated and FSH-V-stimulated donor does via the uterine flushing, and subsequently transferred into the uterine horn of PMSG-stimulated recipient does to obtain kids after undergoing pregnancy period. This study was conducted according to following specific objectives:

a) To develop a suitable protocol for the collection and transfer of goat embryos.

b) To examine the survivability of goat embryos after ET.

c) To produce goat kids through ET.

d) To evaluate the effects ET recipients on ET performance.

e) To compare the effects of different hormones on goat superovulation responses.

f) To compare different ET localities effects on goat superovulation responses and subsequent embryo transfer performance.

g) To compare the performance of donor does with different numbers of flushing cycle.

Chapter 2

2.0 REVIEW OF LITERATURE

Chapter 2

2.0 REVIEW OF LITERATURE

2.1 BACKGROUND

George John Romanes (1848–1894) is the first researcher to attempt embryo transfer in the world, albeit unsuccessful, who had preceded those of Heape cited by Biggers and Walter, 1991. Walter Heape (1855–1929) reported the first live young rabbits born via embryo transfer in April 1890 and he is considered the father of embryo transfer (Betteridge, 1981; Biggers and Walter, 1991; Heap, 1992). The first recorded the use of embryo transfer in farm species was at the Agricultural and Mechanical College of Texas, by Warwick, Berry and Horlacher. In 1932 and 1933, their research group used the technique in both sheep and goats to investigate the causes of *in utero* loss of hybrids between these species (Warwick *et al.,* 1934; Warwick and Berry, 1949). Since then, many research activities on embryo transfer were made. Embryo transfer is now used as a tool in livestock breeding industry. Table 2.1 shows the milestones of embryo transfer in animals.

Table 2.1: Timeline of selected milestones of embryo transfer

Year	Animal	Author	Significant finding
1891	Rabbit	Heape Walter	The first successful production of live young by embryo transfer was performed in rabbits.
1933	Rat	Nicholas	First successful embryos transfer in rat.
1934	Sheep	Warwick *et al.*	First successful embryos transfer in sheep.

(Continued)

(Continued)

Year	Animal	Author	Significant finding
1949	Goat	Warwick and Berry	First successful embryos transfer in goat.
1949	Cattle	Umbaugh	First successful embryos transfer in cattle.
1950	Rabbit	Chang	Success with cooling rabbit embryos for storage was followed up with freezing work.
1950	Pig	Kvasnitski	First successful embryo transfer in pig.
1958	Cattle	Dziuk *et al.*	Develop methods of superovulating cattle, collecting and transferring their embryos through the cervix, handling embryos pending transfer, and synchronising the oestrous cycles of donors and recipients.
1964	Cattle	Mutter *et al*.	The first report of a calf born from non-surgical embryo transfer.
1968	Pig	Polge and Day	Pigs following nonsurgical, transcervical embryo transfer were reported.
1971	Pig and sheep	Baker and Dziuk, Wrathall *et al*.; Baker *et al*.;	Pig and sheep embryos were the first species to be successfully transported over long distances.
1974	Mouse	Whittingham and Whitten	The potential for transporting frozen embryos was demonstrated in mice.
1973	Cattle	Wilmut and Rowson	First calf born following transfer of a frozen-thawed embryo.
1974	Horse	Oguri and Tsutsumi	The first foal produced by embryo transfer was born.
1975	Pig	Curnock *et al*.	Using embryo transfer to introduce new stock into pathogen-free pig herds.
1975	Farm animals	Phillippo and Rowson	Using PGF2α and its analogues to synchronise estrus in farm animals.

(Continued)

(Continued)

Year	Animal	Author	Significant finding
1978	Human	Steptoe and Edwards	The first transfer of embryo from human resulting in birth.
1982	Horse	Yamamoto *et al.*	The first foal to result from the transfer of a frozen-thawed embryo was born.
1986	Sheep	Willadsen	Producing the first 'cloned' lambs by nuclear transfer.
1987	Cattle	Prather *et al.*	Produce live young following nuclear transfer from blastomeres in cattle.
1988	Rabbit	Stice and Robl	Produce live young following nuclear transfer from blastomeres in rabbit.
1996	Goat	Keskintepe *et al.*	The first to report goat development to the blastocyst stage *in vitro* and obtained offspring following uterine transfer.
1997	Goat	Shamsul	Production of two sets of twins goat after embryo transfer in Malaysia.
1998	Cattle	Wells *et al.*	Production of cloned calves following nuclear transfer with cultured adult mural granulosa cells.
2000	Pig	Polejaeva and Campbell	Cloned pigs produced by nuclear transfer from adult somatic cells
2001	Goat	El-Gayar and Holtz	The first successful transfer of goat embryos using open pulled straw (OPS) technique.
2012	Goat	Shariffah	Pregnancy rate of 70% after embryo transfer detected 60 days using ultrasound scanning at University of Malaya Farm.

2.2 MULTIPLE OVULATION AND EMBRYO TRANSFER (MOET) IN GOAT

2.2.1 Oestrus Synchronisation

Oestrus synchronisation (ES) in goats is achieved by control of the luteal phase of the oestrous cycle, either by providing exogenous progesterone or by inducing premature luteolysis. The latter approach is not applicable during seasonal anoestrus, whereas exogenous progesterone in combination with gonadotrophin can be used to induce and synchronise oestrus inanovulatory does (Wildeus, 2004). Controlled internal drug release device (CIDR) was developed in early 1980s and serve as an alternative method of administering exogenous progesterone for oestrus synchronization (Wheaton *et al*., 1993; Godfrey *et al*., 1999). The control of oestrus and ovulation in farm animals remains the basis and a prerequisite for the success of controlled breeding. Oestrus synchronisation in goat has been extensively applied in order to achieve an acceptable oestrus responses by using progestagen combined with PMSG administration 48 hours before or at progestagen withdrawal (Greyling and Niekerk, 1987; Baril *et al*., 1993b).

2.2.2 Superovulation

Goats can be synchronised for breeding at the convenience and advantage of the producer with exogenous hormones like progesterone or analogues of progesterone, in association with pregnant mare serum gonadotrophin (PMSG), human chorionic gonadotrophin (hCG), follicular stimulating hormone (FSH) or injections of cycling does with luteolytic agents like cloprostenol which is a prostaglandin analogue. However, some problems are found associated with PMSG-induced superovulations which are a high number of non-ovulated follicles, early regression of CL, short or irregular oestrous cycles and

potential risk of embryo expulsion (Amoah and Gelaye, 1990). PMSG has the advantage of a lower cost and single-dose protocol, but the variability of responses obtained restricts its use (Pintado *et al.,* 1997). A combination of PMSG and human chorionic gonadotrphin (hCG) has also been widely used to superovulate does (Medan *et al*., 2003). FSH is a better choice of hormone for superovulating does as it provides more oocytes than PMSG. A number of experiments have been performed to compare the superovulatory responses between FSH and PMSG, the evidence favours the use of FSH than PMSG in goat (Mahmood *et al.,* 1991, Abdullah *et al.,* 2008). In their studies, Mahmood *et al*. (1991) reported that average number of recovery of embryos was significantly higher with FSH-P (4.72) than with PMSG (2.50) treatment. Therefore, the importance of moderating the hormonal stimulation protocols based on physiological considerations to optimise the yield of high quality of embryos (Sirard *et al.,* 2006).

2.2.3 Fertilisation

Sperm accumulate in the isthmus of the oviduct for 6-12 hours after natural mating or AI. At the time of ovulation, sperm capable of fertilisation move up to the ampullary-isthmic junction of the oviduct (Hunter and Wilmut, 1983; Wilmut and Hunter, 1984). In goats, asynchrony of oestrus has been commonly observed following progestagen withdrawal in FSH-treated females. The onset of oestrus in Angora, Alpine and Saanen goats has been reported to range from 24 to 54 hours following intravaginal progestagen withdrawal. The does with the shortest interval from intravaginal sponge withdrawal to the occurrence of oestrus recorded a higher ovulation rate, compared to does taking a longer time to exhibit behavioural signs of oestrus. This large variation in the time interval from

progestagen withdrawal to the onset of the induced oestrus period indicates that the time of ovulation cannot be predicted based only on the onset of oestrus. Poor fertilisation rates result, especially in fixed-time AI, as a result of poor synchronisation of oestrus and ovulation (Baril *et al.,* 1989; Baril and Vallet, 1990). Poorer synchronisation efficiency of ovulation is usually observed following superovulation in goats, compared to sheep. It has been reported that in goats only 8.8% ovulations occurred 50 hours following intravaginal sponge withdrawal, while 88% of the ovulations occurred from between 50 to 80 hours, a clear indication that most goats in a fixed-time AI programme (e.g. 36 hours and 48 hours) will be inseminated too early with respect to the time of ovulation. In goats, it has also been reported that the distribution of ovulation within females can be attributed to the time interval from the onset of oestrus to the onset of the pre-ovulatory LH surge, as well as the interval from the first to the last ovulation (Cognie *et al.,* 2003; Gonzalez-Bulnes *et al.,* 2004).

2.2.4 Embryo Recovery

Several methods have been used to recover embryos from the goats, such as surgical (Nowshari and Holtz, 1993), non-surgical (Greyling *et al.,* 2002), laparoscopic (Flores-Foxworth *et al*., 1992) and transcervical (Sohnrey and Holtz, 2000). Now, the surgical embryo collection is the most commonly utilised method for goat embryo flushing programmes, the embryo recovery rate from 60-90%. However, the commonly applied surgical approach to embryo collection in goats has several disadvantages, such as the stress of anaesthesia and surgery, postoperative adhesions limiting the number of possible interventions and high expenses (Holtz, 1996). The post-operative adhesion has

been identified to be the main factor leading to a reduction in ovulation rate and embryo yield following repeated superovulation treatment (Al-kamali *et al*., 1985; Cognie, 1999). Non-surgical methods of embryo collection such as the use of laparoscopy and the transcervical passage of a catheter by mechanical dilation of the cervix or ripening of the cervix with PGF2α, or oestradiol have been performed with success in sheep and goats (Mckelvey *et al.,* 1985; Pereira *et al.,* 1998; Wulster-radcliffe *et al.,* 1999). Laparoscopic embryo collection has the advantage of leading to fewer adhesions and putting less strain on the animal compared with the surgical collection, however, the approach requires special instruments and skilled personnel (Pereira *et al.,* 1998; Suyadi *et al.,* 2000). Baril *et al*. (1989) reported that they could collect the embryos more than 7 times using the laparoscopy method in one goat compared with surgical method with the embryo recovery rates range from 60 to 78.7% by laparoscopic (Baril *et al.,* 1989, Flores-Foxworth *et al.,* 1992). Laparoscopic approach puts less strain on the animals, but requires special instruments and skill. Transcervical embryo collection has repeatedly been attempted in goats and sometimes led to a degree of success (BonDurant *et al.,* 1984; Nagashima *et al.,* 1987; Bessoudo *et al.,* 1988; Flores-Foxworth *et al.,* 1992). As previously stated, transcervical embryo collection has several advantages over surgical and laparoscopic embryo collection procedures. Using this approach embryo recovery rates ranging from 60 to 80% have been obtained in goats (Nowshari *et al.,* 1995; Suyadi *et al.,* 2000; Holtz, 2005) compared with other methods, less trauma to the animal, no need for sedating the animal and no limitation to the number of times a donor can be flushed. This procedure hence holds the potential for more popular utilisation in goats, if further investigation could improve the efficiency this procedure (Lehloenya *et al*.,

2008).

2.2.5 Embryo Transfer

2.2.5.1 Method of embryo transfer in goat

Embryos have been transferred in goats by surgical, laparoscopic and transcervical methods with success (Bessoudo *et al.,* 1988; Flores-Foxworth *et al.,* 1992; Besenfelder *et al.,* 1994). Normally, most researchers chose the surgical method to transfer the embryo into the oviduct or uterine horn. The reproductive tract being exteriorised via a mid-ventral laparotomy, which allows for the inspection of the ovaries for presence of corpus luteum (Armstrong and Evans, 1983; Kiessling *et al.,* 1986; Li *et al.,* 1990; Selgrath *et al.,* 1990; Yuswiati and Holtz, 1990; Wallace, 1992; Guignot *et al.,* 2006). However, the surgical method is really cause major post-surgical adhesions, therefore, may be limiting for repeatedly conducted surgery on the same doe. Only the tip of the uterine horn ipsilateral to the ovary displaying an ovulation site needs to be exteriorised (Holtz, 2005) or using the laparoscopic method to transfer the embryo. Compare with the surgical method, it is safe easy and quick to perform and it creates the opportunity to visually confirm the presence and quality of corpus luteum before transfer. Also the conception rate is similar (70 to 75%) to that obtained using mid-ventral laparotomy (McKelvey *et al.,* 1985; Flores-Foxworth *et al.,* 1992; Baril *et al.,* 1993a; McMillan and Hall, 1994; Ishwar and Memon 1996; Cognie, 1999). Other transcervical method is less popular, because even so the pregnancy rate is no significant have been found, but it has two reasons cause it not popular: does not allow for the confirmation of the presence of corpus luteum. Moreover, trancervical transfers have been reported to induce

contractions of the cervix and the uterus, which result in the rejection of the transferred embryos (Rowson, 1971; Flores-Foxworth *et al.*, 1992; Wallace, 1992; Cognie, 1999).

2.2.5.2 Factors influencing the survivability of transferred goat embryos

2.2.5.2.1 Site of embryo transfer

Normally, embryos are transferred into the oviduct or uterine horn, depending on the age or stage of development of the embryos being transferred. Early embryonal stages (up to 8 or 16 blastomeres) are to be transferred to the oviduct (Armstrong and Evans, 1983; Wallace, 1992; Wang *et al*., 2003; Holtz, 2005). In goat and sheep, it was found that the low survival rate of an early stage (form 1 cell to 8 cell) embryo transferred into the uterine horn, the early embryo stage better transferred to the oviducts of recipients, and embryos which had developed beyond 8 cells should be transferred to the uterus. Because the uterus does not provide an environment suitable for the survival and development of embryos of 2 and 4 cells (Averill and Rowson, 1958; Moore and Shelton, 1964; Armstrong and Evans, 1983; Ishwar and Memon, 1996). For uterine horn transfer, flushing the embryo after 6-8 days after donors are seen in oestrus following natural or artificial breeding (Nowshari and Holtz, 1993; Ishwar and Memon, 1996; Guignot *et al*., 2006). In goats, embryos are mostly transferred into the oviduct or uterine horn ipsilateral to the ovary, with at least one normal corpus luteum (Moore and Eppleston, 1979; Bessoudo *et al.*, 1988; Stefani *et al.*, 1990; Nowshari and Holtz, 1993; Besenfelder *et al.*, 1994; El-Gayar and Holtz, 2001; Holtz, 2005; Guignot *et al.*, 2006).

2.2.5.2.2 Number of embryos transfer

In goat and sheep, the survivability of the embryos is higher when 2 embryos are transferred compared with of one or 3 embryos. For 2 embryos transfer, over 80% of recipients carried pregnancies to term, with approximately two-thirds of these giving births to twins, compared with more embryos (>5) transferred, the proportion of recipients becoming pregnant was the same when receiving 5 embryos or less. But the ovum survival rate following transfer of 5 embryos were lower (Moore, 1974; Armstrong and Evans, 1983; Tervit *et al.*, 1986; Ishwar and Memon, 1996; El-Gayar and Holtz, 2001). The survival of embryos transferred as twins was significantly higher when both embryos were transferred to the same oviduct (unilateral transfer) than when one was transferred to each oviduct (bilateral transfer) (Armstrong and Evans, 1983; Ishwar and Memon, 1996).

2.2.5.2.3 Age and quality of embryos transfer

The age and quality of the embryo has a significant effect on the subsequent survival rate. Embryos are generally collected 48-60, 60-72 and 72-84 hours after donors are seen in oestrus following natural or artificial breeding. There was an increase in number of ewes which lambed and lambs born with increase in age of transferred embryos in the case of sheep (Moore and Shelton, 1964). In contrast to these findings, Armstrong reported a decrease in number of transferable embryos as the interval from estrus to flushing was increased in goats. The most dramatic decrease occurred when flushing was delayed beyond Day 5. Luteal regression was observed in 5 of the 7 goats flushed at 6-8 days, and in one of 3 flushed on day 5 (Armstrong and Evans, 1983; Ishwar and Memon, 1996). At present, embryos are generally collected on days 7 to 8 following ovulation, at this time

the recovered embryos are expected to be at the morula to blastocyst stage in goat (Yang *et al.,* 1991; Gordon, 1997). In the sheep, the normal expectation was that embryo would be at the morula and blastocyst stages of development respectively. And there was no significant overall effect of day of embryo collection on subsequent survival rate. However, advanced (blastocyst) embryos collection on day 5 had a higher survival rate than retarded (morula) embryos collected on day 6, with normal stage embryos (day 5, morula; day 6 blastocyst) have intermediate survival rate (Bari *et al.,* 2003). For the embryo quality, normally it is dependent on the age of embryos, as it is based on the morphological appearance and the stage of development. Compared with Grades 1 and 2 embryos, the Grades 3 and 4 embryos survival rate was much lower (Breuel *et al.,* 1991; Bari *et al.,* 2003).

2.3 FACTORS AFFECTING THE EFFICIENCY OF MOET

Factors causing high viability are the major constraint for MOET programme in goat being classified either as extrinsic such as season, nutrition, different exogenous superovulation techniques used, fertilisation, repeated superovulation and embryo recovered; or intrinsic factors such as age, breed, reproductive status.

2.3.1 Extrinsic Factors Affecting MOET in Goat

2.3.1.1 Seasonal effects on MOET

Seasonal breeding is a survival strategy adopted by many wild mammals to ensure that their offspring are born at the most favourable time of the year. In sheep, which have been domesticated for almost 10,000 years, a seasonal pattern of reproduction persists in

responses to an endogenous rhythm that is entrained by photoperiod. Ewes in temperate regions respond to decreasing day-length in autumn by increasing the activity of the GnRH pulse generator and show oestrus and ovulation throughout the winter months (Wheeler and Land, 1977). As day-length increases in spring, there is a gradual slowing of GnRH pulsatility until a pre-ovulatory LH surge can no longer be generated and oestrus cyclicity ceases (Bittman *et al.,* 1985). Ovarian follicular development does not, however, cease (Cahill and Mauleon, 1980) and successive waves of follicles continue to develop throughout anoestrus (Bartlewski *et al.,* 1998).

The goat is seasonal polyestrous animal, the start oestrus time and frequency which depend on the breed and district. Normally, the oestrus seasonal of goat like the sheep, which major appear in spring and autumn, special vigorous in autumn, and multi-oestrus in each breeding season (Restall, 1992).Some of species of goat oestrus year-around in the suitable condition, most of goats species which live in the torrid zone and subtropical zone even some species live in the polar zone have this feature. Seasonal variation in reproductive in Boer goat with a peak incidence turn up in autumn, the lowest appear in deep spring and mid-summer, however, it never appear the anoestrus period (Greyling and Niekerk, 1987). During the late compared to peak breeding season, there is an increased incidence of fertilisation failure as a possible consequence of seasonal shifts in LH secretion and (or) associated effects on follicular function. Frozen–thawed embryos produced at contrasting stages of the breeding season are equally viable *in vivo* but those produced during the late, as opposed to the peak breeding season have lower viability following *in vitro* culture (Mitchell *et al*., 2002). Gonzalez-Bulnes *et al.* (2003) showed that there were no significant differences in the ovarian responses to FSH

treatment between the breeding and no breeding seasons. However, the recovery rate tended to be higher in no breeding season. Follicular dominance is more profound during the breeding season than during the anoestrus period.

2.3.1.2 Nutritional effect on MOET

Nutritional status and dietary intake of the animal are known to influence reproductive performance through a network of complex relationships and interactions, which include hormone production, gametogenesis, fertilisation and early embryonic development in ruminants (O'Callaghan and Boland, 1999; Boland *et al.,* 2001; Peura *et al.,* 2003). Nutrition affects all aspects of the chain of reproductive events from gametogenesis to puberty in both males and females. The reason for this close association between nutrition and reproduction is to ensure that reproduction is very closely aligned with the food supply (Scaramuzzi *et al.,* 2006). In sheep and goat, the low level of nutrition reduce the ovulation rate, low intakes after mating induced a higher rate of ova wastage (Smith, 1991; Mani *et al.,*1992), Nevertheless, Parr *et al.* (1987) reported that for Merino ewes, overfeeding during the first 2 weeks of pregnancy was also associated with a significant reduction in pregnancy rate.

As shown in Table 2.2, the relationship between nutrition and reproduction is through energy balance. When the animal in "negative energy balance" state, it will use their energy stores (glycogen, triglycerides and protein) to meet the energy deficit. Also, when the animal in "positive energy balance" state, it will store the excess nutrients

Table2.2: Some known associations between energy balance and reproduction

Metabolic state	Metabolic consequence	Effects on reproduction

Negative energy balance	– Weight loss – Fat stores depleted – Muscle wasting – Hypoinsulinemia – Hypoglycaemia – Elevated βOH butyrates and NEFA – Elevated GH – Low Leptin – Reduced metabolic heat – Suppressed IGF system – Elevated urea	– Inhibition of GnRH secretion by the hypothalamus – Absence of LH pulses – Low FSH concentrations – Inhibition of folliculogenesis – Low oestradiol – High negative feedback sensitivity – Anovulation – Anoestrus – Delayed puberty
Energy balance	– Weight maintained – Fat stores maintained – Normal insulin – Normoglycaemia – Low NEFA and βOH butyrate – Normal GH – Normal Leptin – Normal IGF system – Normal urea	– Normal GnRH secretion by the hypothalamus – Normal LH pulsatility – Normal FSH concentrations – Normal folliculogenesis – Normal oestradiol and inhibin – Normal negative feedback – Ovulation – Oestrus – Ovulation rate below natural maximum
Positive energy balance	– Long-term weight gain – Fat stores increased – Hyperinsulinemia – Hyperglycaemia – Low NEFA and βOH butyrate – Low GH – Elevated leptin – Increased metabolic heat – Stimulated IGF system – Urea normal but can be high ifdietary nitrogen is high	– Normal GnRH secretion by the hypothalamus – Normal LH pulsatility – Increased FSH concentrations – Reduced oestradiol – Reduced negative feedback – Ovulation – Oestrus – Maximum natural ovulation rate – Advanced puberty

(glycogen and triglycerides) and/or disperse the excess nutrients as metabolic heat (Scaramuzzi, 2006).

The animal body condition influences the quality of oocyte. In cattle, restricting the energy intake before the animals were killed enhanced the subsequent *in vitro* development of the oocytes (McEvoy *et al.,* 1997; Nolan *et al.,* 1998; Armstrong, 2001). The effect of feeding level on oocyte quality is dependent on the body condition of animal, with the high level of feeding being beneficial to oocytes from animals of low body condition, but detrimental to oocytes from animal of moderately high body condition.(Adamiak *et al,*. 2005).

Therefore, these findings indicate that some of the effects of nutrition on reproduction may influence the oocyte development and early embryo development or uterine environment. This is why nutritional management of donor goat in programmes of superovulation need to be adapted to optimise the production of oocytes and embryos (Scaramuzzi and Murray, 1994).

2.3.1.3 Different exogenous superovulation techniques used effect on MOET

The most relevant drawback for the MOET programme is the lack of an effective and consistent means of superovulating donor animals. In goats, superovulatory treatment typically consists of a combination of oestrous cycle control (usually involving application of progestagen implants) with an elevated dose of a gonadotrophin, to induce the ovary to release more than the typical number of oocytes (Holtz, 2005). Different exogenous supervoulation techniques used might also lead to variation in superovulation responses as it determines the rate of gonadotrophin absorption (Dobbs *et al.,* 1994). Nowadays, the gonadotrophin preparations most frequently used are pregnant mare serum gonadotrophin (PMSG/eCG), porcine follicle stimulating hormone (pFSH), horse

anterior pituitary extract (HAP) and human menopausal gonadotrophin (hMG) (Wallace, 1992).The first gonadotrophin to be used in a superovulation programme for goats was eCG. This hormone was administered as a single injection one to two days before or at progestagen treatment termination, as eCG is known to have a long half-life, however, in many cases did not deliver the anticipated responses (Amoah and Galaye, 1990; Holtz, 1996; Saharrea *et al.*, 1998; Cognie, 1999). This might be associated with the rapid degradation of eCG in goats; its half-life being only 10–15 hours (Holtz, 1996), which is several times shorter than in cows. Maybe the long half-life lead to the production of a large number of ovarian follicles which fail to ovulate. These unovulatory follicles are then often associated with lower quality embryos recovered following ovulation (Boland *et al.,* 1978; Saumande *et al.,* 1984). Also, in other species, the large unovulatory follicles of embryo recovery has also been reported treated with eCG (Monniaux *et al.,* 1983; Kafi and McGowan, 1997; Naqvi and Gulyani, 1998).

Currently the most commonly used FSH preparations in goat MOET programmes are ovine (oFSH) and porcine (pFSH) produced FSH (Baril and Vallet, 1990; Cognie, 1999). Follicle stimulating hormone (FSH), usually of porcine origin (pFSH), proved to be more efficacious than eCG (Armstrong and Evans, 1983; Mahmood *et al.,* 1991; Nowshari *et al.,* 1992). Compare with the eCG, the half-life of pFSH in goats is only 5 hours (Demoustier *et al.,* 1988), Nowshari *et al.* (1995) proved that it contains an appropriate admixture of luteinising hormone (LH) which contains in the range of 40% does not only provide the best superovulatory responses but also superior embryo viability. Because the short half-life compare with eCG, the preparation of FSH being normally administered twice daily at 12 hours intervals, over a period of 3 to 4 days, in

goats (Demoustier *et al.,* 1988; Pendleton *et al.,* 1992; Rosnina *et al.,* 1992; Gordon, 1997; Holtz, 2005). These reports satisfactory ovulation rates ranging between 8.4±0.9 and 28.7±2.3 have been recorded in goats superovulated with FSH (Senn and Richardson, 1992; Rosnina *et al.,* 1992; Ishwar and Memon, 1996; Baril, 1995; Holtz, 1996; Greyling *et al.,* 2002).

2.3.1.4 Repeated superovulation and embryo recovery effect on MOET

The MOET programme can accelerate the genetic progress in goat. Superovulation and recovery of embryos could be repeated from same donor doe, which can be a feasible way to optimise *in vivo* embryo production in females that have high genetic value (Forcada *et al.,* 2010). However, repeated superovulation in small ruminants in the past has led to undesirable side effects. Repeated superovulation with pFSH in goats has been reported to reduce the number of ovulations and embryos recovered, the quality of embryo, as well the number of transferable embryos (Nuti *et al.,* 1987; Baril *et al.,* 1989; Beckers *et al.,* 1990). In the sheep, beyond that reduced the ovulation rate, the oestrus responses and the number of does ovulating were reduced (Al-kamali *et al.,* 1985; Fuki *et al.,* 1985). There are three main side effects of repeated superovulation in goat MOET programme. Firstly, the refractoriness of the ovaries if superovulation was repeated within an interval of 2 to 6 months (Willett and Buckner, 1953; Al-kamali *et al.,* 1985; Nuti *et al.,* 1987; Brebion *et al.,* 1992). Secondly, the reduction in ovulation rate and the number of females responding to repeated superovulation was that gonadotrophin antibodies are formed following successive superovulation (Holtz, 2005). The Anti-eCG antibodies produced in goats have been indicated to have a negative effect on reproduction, especially when

fixed-time AI is performed. The high concentration of Anti-eCG antibodies was correlated with a decrease in fertility. The reduction in fertility is believed to arise from the alteration in the time of the occurrence of the expected oestrus (Roy *et al.,* 1999). The delay in the preovulatory LH surge and time of ovulation observed following repeated eCG treatment has also been associated with the formation of anti-eCG antibodies (Herve *et al.,* 2004). Lastly, the embryos recovered method also affect the result of flushing (refer to Section 2.2.4, i.e., embryo recovery).

2.3.1.5 Fertilisation effect on MOET

Normally, for the donor does, oocyte fertilisation is carried out using natural mating and AI (artificial insemination) (Moore, 1982). The fail of fertilisation after AI for sheep is more than that of goat that maybe due to difficulty to pass the AI gun through the cervix in the former. For the goats, the AI gun containing the sperm in the straw can pass easily through the cervix into the uterus (Moore and Eppleston, 1979).

Successful fertilisation is achieved with proper oestrus synchronisation in the donor doe and the mating method. For Angora, Alpine and Saanen goats, the oestrus period range from 24 to 54 hours after intravaginal progestagen withdrawal. Compared to does taking a longer time to exhibit behavioural signs of oestrus, the does with the shortest interval from intravaginal sponge withdrawal to the occurrence of oestrus recorded a higher ovulation rate. Poor fertilisation, especially in fixed-time AI, is the result of poor synchronisation of oestrus and ovulation (Baril *et al.,* 1989; Baril and Vallet, 1990). Akinlosotu and Wilder (1993) found that injection the GnRH after intravaginal sponge withdrawal could reduce the ovulated period, then increase the ovulation rate. It

was reported that not only the ovulation rate increased by GnRH treatment following superovulation with eCG, but also the ovulation time was synchronized, leading to 91% of the ovulations occurring between 36 and 48 hours following sponge removal. In goats treated with only eCG, ovulations continued to occur until up to 77 hours following intravaginal sponge withdrawal. This observation indicates that the fertilisation rate could be improved in goats superovulated with FSH by treating the animals with GnRH, as the occurrence of ovulation could be synchronised (Cameron *et al.,* 1988). For most species, a lack of sperm recovery and sperm numbers in the uterus and oviducts (site of fertilisation) following superovulation, with either FSH or eCG cause the fertilisation failure (Evans and Armstrong, 1984). For goat, impeded sperm transport following superovulation has also been a result of premature and early ovulation, which lead to an increase in circulating progesterone concentrations during the preovulatory period. The sperm damage reduces the fertilisation rate whereby the time the sperm reach the oviduct was later, thus causing damage the sperm and consequently the oocyte released earlier will not be fertilised (Moor *et al.,* 1984; Cameron *et al.,* 1988). The fertilisation failure could also be ascribed to be due to abnormal maturation of the oocytes following superovulation. This condition contributes to major embryonic loss following superovulation, as some of these activated oocytes will be left as luteinised follicles, while others will be ovulated as old eggs and will end up being abnormal embryos (Moor *et al.,* 1984; Kumar *et al.,* 1990, 1991).

2.3.2 Intrinsic Factors Affecting MOET in Goat

2.3.2.1 Age of the goat effect on MOET

Multiple ovulation and embryo transfer (MOET) is utilised to accelerate the genetic improvement in goat breeding. The effect of age of the donors on the ovarian responses to superovulation is one of important effect for MOET programme. Most studies have been comparing prepubertal to adult animals. There is a general agreement that pre-pubertal and young (age, 1 to 2 years) animals can be superovulated and these follicles are also sensitive to gonadotrophin stimulation (Donaldson, 1984; Driancourt *et al*., 1990; Rangel-Santos *et al*., 1991; Hasler, 1992; Kuhholzer and Brem, 1999). Age could contribute greatly to the variation in ovarian responses to superovulation between animals within the same group, as animals of different ages have been shown to have different physiological needs and respond differently (Jainudeen *et al*., 2000).

For goats, there is limited information relating to the effect of age on the ovarian responses to superovulation, compared to other ruminants. When evaluating the effect of age on oestrus responses following superovulation, younger does recorded a longer time interval from CIDR removal to the onset of oestrus. Similar results have been reported in cattle and goats (Drion *et al*., 2001; Lehloenya and Greyling, 2010). For the young does it was the first time to be treated with exogenous hormones, thus, these does may have responded slower and was less sensitive to the exogenous hormones due to high sensitivity to the negative effects of steroids. Also, a poorer response to superovulation, a lower ovulation and fertilisation rates, leads to 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).

Compared the adult females, the old age females generally become less reproductively competent and largely due to decreasing uterine health and oocyte

viability (Carnevale *et al.,* 1993, 1997; Morris and Allen, 2002; Morel *et al.,* 2005). Morel *et al.* (2010) found that a slower final growth rate of the pre-ovulatory follicle or a longer time interval from the achievement of maximum pre-ovulatory follicle diameter to ovulation in old animals related to reduction in gonadotrophin secretion and/or altered secretion of insulin-like growth factors (IGF).

2.3.2.2 Breed of the goat effect on MOET

Breed is widely recognised as a major factor of variation (Torres *et al.,* 1987). Different breeds or genotypes have different responsiveness to gonadotrophins in MOET programme, such as mice (Spearow, 1988), sheep (Vivanco *et al.,* 1994), cattle (Critser *et al.,* 1979). In a very exhaustive study using over 9000 superovulated sheep, the breed factor accounted for approximately 30% of the variability in the embryo yields obtained in responses to FSH treatments (Vivanco *et al.,* 1994). Cahill *et al*. (1979) reported that most of the differences in superovulatory responses were related to the different prolificacy of the breeds used in MOET. Bindon (1971) and Smith (1976) found that highly prolific breeds having a greater response to exogenous stimulation. Thus, it has become important to establish a reliable method of superovulation for each individual breed as the first step toward the establishment of reliable least variable embryo transfer programmes (Ammoun *et al.,* 2006). Nuti *et al*. (1987) compared that Alpine and Nubian does in the first step (oestrus synchronisation) of a MOET programme, 87% of the Nubian goats came into oestrus 36 hours following sponge removal, while only 50% of the Alpine goats showed signs of oestrus. The onset of oestrus has a big influence on the ovulation rate following superovulation. The rates of follicular growth and function may

be the underlying reason for the different responses to exogenous hormone observed between breeds (Bindon *et al.,* 1986; Bartlewski *et al.,* 1999). In goats it has been reported that a higher number of embryos (average of 10.1) were recovered in Alpine does, compared to Angora does (average of 7.5) (Baril *et al.,* 1989). Ultimately, the conclusive factor is gene, Dufour *et al.* (2000) found that the *fec* gene have a higher ovarian follicle selection rate. Therefore, a lower ovulation rate following superovulation has been recorded in this breed, due to the high atretic rate recorded among recruited follicles. On the other hand, ewes without the *fec* gene have shown less follicular atresia, making more follicles available to be recruited and proceeding to the ovulation stage, hence leading to a high ovulation rate following superovulation.

2.3.2.3 Reproductive status effect on MOET

In the MOET programme, the reproductive status is the most important factor for this programme, nevertheless, the associated with the ovary should be given primary importance among priorities.

Firstly, the ovarian responses following superovulation, recently, the accuracy of transrectal real-time ultrasonography (RTU) scanning technique to detect ovarian structures (follicles and corpus luteum) of goat compared with the laparoscopy and laparotomy techniques, RTU technique non-invasive the animal and obtained the number of follicles ≥3 mm (Simoes *et al.,* 2005).

The growth of ovarian antral follicles to periovulatory sizes of ≥5 mm in diameter was seen at all stages of anoestrus. An average of four waves of follicular development (follicles growing from 3 to ≥5 mm in diameter before regression) with a periodicity of 4

days were recorded during each of the three scanning periods. There was a close temporal relationship between days of follicular wave emergence and peaks of successive FSH fluctuations. Ewes entering anoestrus late exceeded ewes that became anoestrus early in numbers of large (≥5 mm in diameter) ovarian antral follicles and maximum follicle diameter. Peak concentrations of transient FSH increases were higher (Bartelewski *et al.*, 1999).

In 15 of 20 (75%) goat obtained that interovulatory intervals include 4 follicular wave, the mean days of emergence of waves 1 to 4 were days −1, 4, 8 and 13, respectively, interval 4 days (Ginther and Kot, 1994). For the 4 waves, the first wave, second wave, third wave and the ovulate wave appear in days −1, 7, 11 and 13, respectively (De Castro *et al.,* 1999). In goats with three follicular waves, the number of 3mm follicles peaked on days 0, 7 and 11, whereas in goats with four follicular waves, the number of 3 mm follicles peaked on days −1, 5, 11 and 15 (Medan *et al.*, 2003).

In goat oestrus period, individual follicle profiles (follicle growing from 3 to ≥5 mm in diameter) for representative animals and accompanying concentrations of FSH, ir-inhibin, inhibin A, oestradiol-17β, and progesterone are depicted in Figures 2.1 and 2.2 (Medan *et al.,* 2003). The concentrations of FSH rise 2 to 3 days before the emergence of follicular wave, temporal associations between FSH peaks and the emergence of follicular waves throughout the oestrous cycle have been described for goat (Ginther and Kot, 1994). The number of emerging follicular waves and of identified FSH peak values per goat did not differ. Plasma FSH concentration was highest at the emergence of each wave and decreased as the follicle grew to 5 mm in diameter, plasma levels of ir-inhibin and of inhibin A were low during follicular wave emergence and increased with the

growth of follicles at the time of declining FSH. The duration of the interval between adjacent days of wave emergence (inter-wave intervals) was positively correlated with the duration of the inter-peak interval for FSH fluctuations (Medan *et al.,* 2003).

The oestradiol concentrations were greatest during the growing phase of the first dominant follicle and significantly reduced when the dominant follicle attained its large diameter (Rhodes *et al.,* 1995). It is well established that the secretion of FSH during the oestrous cycle is regulated by both oestradiol and inhibin (Mann *et al.,* 1992). The oestradiol-17β profiles in the present study are characterised by gradual increase from the day of ovulation to day 4 then decreased to the basal level.Oestradiol-17β levels remained low during the rest of the luteal phase, apart from some isolated fluctuations then increased, reaching a peak 2 days before ovulation (Medan *et al.,* 2003). In some early research for cattle and sheep, oestrogens are mainly produced by the dominant follicle of a wave and that subordinate follicles contribute less than 10% of the ovarian oestradiol-17β production. In the present study, although oestradiol-17β was produced by the large follicles of each follicular wave, the first and last waves secreted more oestradiol-17β compared with other mid-luteal waves. This might be attributed to decreased LH release during the mid-luteal phase (Walters *et al.,* 1984; Kawate *et al.,* 2000). The results of their studies suggest that changes in the secretion of inhibin control FSH during the oestrous cycle in goats. Mean plasma FSH started to increase from approximately the time of the end of the growth phase or onset of the static phase of the follicular wave, suggesting that secretion of follicular inhibitor of FSH release declined at that time. New follicular waves emerged within 1 or 2 days of the onset of the static phase of the previous wave, suggesting that the changed secretory activity of follicles in the

static phase permits the increase in FSH secretion that heralds the next follicular wave. The dimeric inhibin A is related to the presence of large follicles and is negatively correlated with FSH concentration, suggesting that inhibin A is a product of differentiated follicles and has an important role in controlling FSH secretion (Medan *et al.,* 2003). Similarly, in rats, inhibin A is at its highest concentration during prooestrus, concomitant with the selection of large follicles (Woodruff *et al.,* 1996). Previous studies demonstrated that whereas oestrogenic large follicles are a major source, both small and large non-oestrogenic follicles contribute significantly to the ovarian secretion of ir-inhibin (Campbell *et al.,* 1991; Mann *et al.,* 1992).The relationship between the pattern of inhibin release, FSH and follicular growth is similar to that reported by Kaneko *et al.* (1995) in cycling cows and cattle (Kaneko *et al.,* 2002).

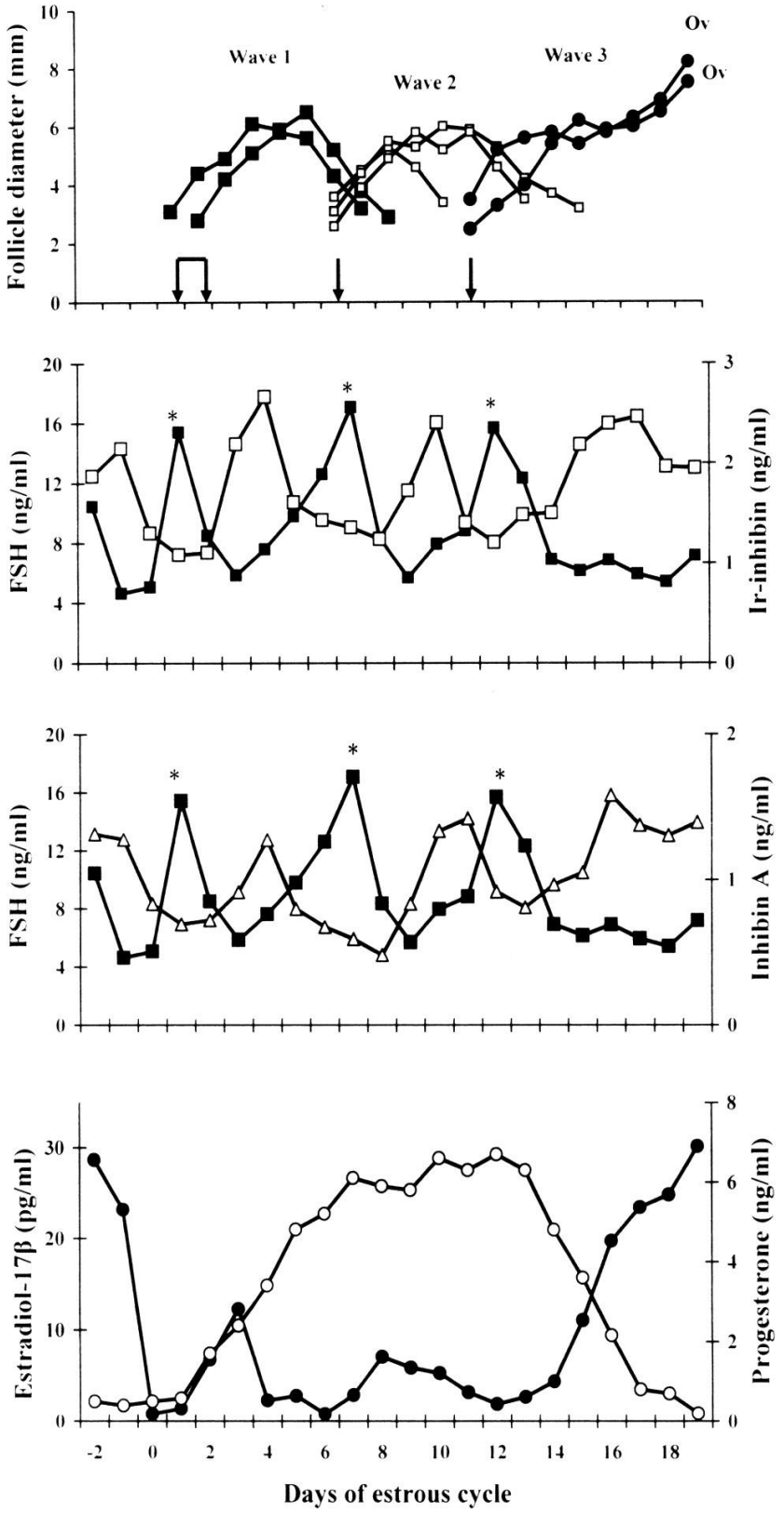

Figure 2.1: Representative pattern of growth and regression of individual follicles during oestrous cycles in a goat with three waves of follicular development and accompanying plasma concentrations of FSH (■), ir-inhibin (□), inhibin A (▵), estradiol-17β (•), and progesterone (○) (Adapted form Medan *et al.,* 2003).

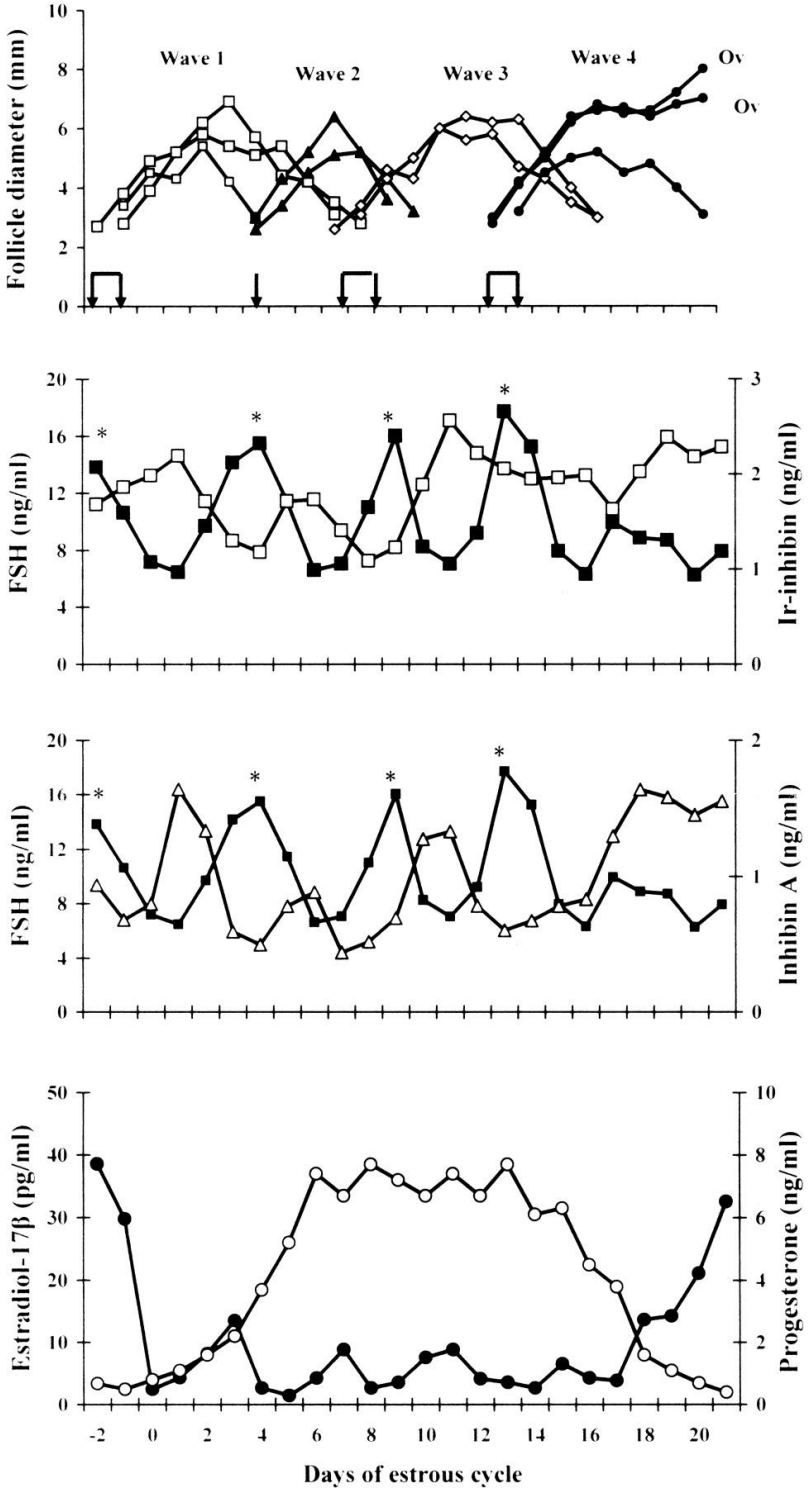

Figure 2.2: Representative pattern of growth and regression of individual follicles during oestrous cycles in a goat with three waves of follicular development and accompanying plasma concentrations of FSH (■), ir-inhibin (□), inhibin A (△), estradiol-17β (•), and progesterone (○) (Adapted form Medan *et al.*, 2003).

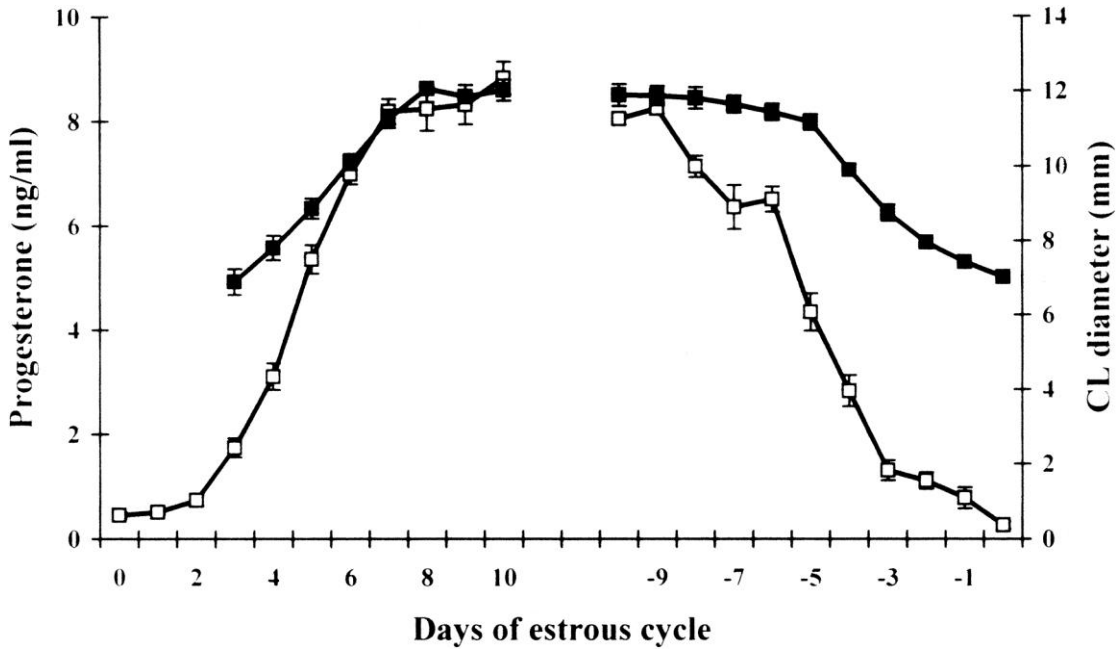

Figure 2.3: Diameter of CL (■) and progesterone concentrations (□) during the estrous cycle in goats (Adapted form Medan *et al.*, 2003).

The CL was detected ultrasonically on day 3 post-ovulation and attained a mean maximum diameter of 13.5+0.8 mm between days 8 and 14 (De Castro *et al.,* 1999). During the early luteal phase, the mean diameter of CL increased in parallel with the mean plasma concentration of progesterone, whereas during the late luteal phase, the plasma concentration of progesterone decreased more rapidly than the CL regression (Medan *et al.*, 2003) (Figure 2.3).

The relationship between higher progesterone levels during the early luteal phase and an acceleration of follicular turnover, when the progesterone concentrations increase early in untreated goats, the emergence of wave 2 was advanced (Menchaca and Rubianes, 2002). The results were same with the cows (Adams *et al.,* 1992) and ewes (Rubianes *et al.,* 1996). For the spontaneous oestrus goats, there are 4 follicle waves in the oestrous

cycle, the mean concentrations of progesterone in the cycle 1 to 5 days evidently were higher than third wave, and the mean oestradiol concentrations lower in cycle 3 to 5 days compared with in the third wave. The second follicle wave appears in forth wave early than third wave (Rubianes and Menchaca, 2003).

The goat growth of ovarian antral follicles not only do activity in traditional reproductive season, but also can been observed in anoestrus season even in gestation period. The growth of ovarian antral follicles to peri-ovulatory sizes of ≥ 5 mm in diameter was seen at all stages of anoestrus. An average of four waves of follicular development (follicles growing from 3 to ≥ 5 mm in diameter before regression) with a periodicity of 4 days were recorded during each of the three scanning periods. There was a close temporal relationship between days of follicular wave emergence and peaks of successive FSH fluctuations. Ewes entering anoestrus late exceeded ewes that became anoestrus early in numbers of large (≥ 5 mm) in diameter. Ovarian antral follicles and maximum follicle diameter (Bartlewski *et al.*, 1999).

Secondly, the type of follicular wave: Ginther and Kot (1994), De Castro *et al.* (1998) and Gonzalez-Bulnes *et al.* (1999) found that the daily ultrasonography in this study indicate that the interovulatory interval in goats is characterised by a wave-like pattern of follicular development, This is similar to previous findings inwhich daily ultrasonic scanning was used in other ruminant species (Ginther *et al.*, 2001). A folliclewave involves the emergence of a group of small antral follicles from which commonly one or two follicles are selected to grow to more than 5 mm in diameter. According to different authors the number of follicular waves ranges between two and five waves per cycle, but the predominant pattern for goats that developed an

interovulatory cycle of normal length (19–22 days) is of four waves (Ginther and Kot, 1994; De Castro *et al.,* 1999; Schwarz and Wierzchos, 2000; Menchaca and Rubianes, 2002). The emergence of waves 1, 2, 3 and 4 (the ovulatory wave) occurs on Days 0, 5–6, 10–11 and around Day 15 post-ovulation, respectively. In goats that developed three follicular waves, wave 2 emerges 1–2 days later and the ovulatory wave emerges 1–2 days earlier. Some of the more frequently observed characteristics of the follicular waves are: (a) the diameter of the largest follicle of a wave differs between waves; generally the largest follicles of waves 2 and 3 attain smaller maximum diameters than both, the largest follicle of wave 1 and the ovulatory follicle (Ginther and Kot, 1994; De Castro *et al.,* 1999; Menchaca and Rubianes, 2002); (b) two or more follicles frequently attain 5 mm or more in diameter per wave (Ginther and Kot, 1994; Schwarz and Wierzchos, 2000; Pinczak *et al.,* 2001); (c) the growth rate between the day of appearance (first day with a size of 3 mm) and the day of maximum diameter is around 1 mm per day (Ginther and Kot, 1994; Gonzalez-Bulnes *et al.,* 1999; Schwarz and Wierzchos, 2000; Pinczak *et al.,* 2001); (d) along with the luteal phase progresses, follicular turnover increases and the inter-wave intervals are shorter than during the early luteal phase (Ginther and Kot, 1994; De Castro *et al.,* 1999); (e) the follicles that do not grow beyond 4 mm during the mid-late luteal phase often are not part of the wave phenomenon, and it is suggested that they represent a dynamic underlying pool (Ginther and Kot, 1994; De Castro *et al.,* 1999); (f) on the day of luteolysis most of the ovulatory follicles are the largest follicles (Ginther and Kot, 1994; De Castro *et al.,* 1999); (g) in most double ovulatory goats the ovulatory follicles emerged as part of the same follicular wave. However, in a few cases also as a part of different waves (Ginther and Kot, 1994); and (h) in most cycles the double ovulations

occur on the same day (Ginther and Kot, 1994).

Thirdly, the factor affecting the follicular wave: many factors affect the follicular wave development, such as: breed, the polyembryony gene, nutrition and so on (Bartlewski *et al.,* 1999; Gibbons *et al.,* 1999; Viñoles *et al.,* 2002). Also the different hormone effect the follicular wave. The wave of FSH concentrations and the emergence of follicle waves are closely related (Bartlewski *et al.,* 1999; Evans *et al.,* 2002). Evans *et al.* (2002) concluded that: (a) transient enhance in FSH concentrations precede the emergence of follicle waves; (b) all follicles ablate on day 4.5 after oestrus advanced the timing of the next peak in FSH concentrations and the numbers of small follicles associated with the development of the second follicular wave; and (c) ablation of the largest follicle resulted in an enhance in the lifespan of the second largest follicle, indicating a regulatory role of large dominant follicles over smaller subordinate follicles.

Waves developing when under continuous and high progesterone influence have a smaller large follicle than those developing when progesterone concentrations are low (Ginther and Kot, 1994). De Castro *et al.* (1999) found that the progesterone concentrations were higher in the 4^{th} follicular wave goats between day 5 and day 10 than in the other goats that only got 2 or 3 follicular waves. The mean progesterone concentrations between days 1 and 5 were higher than in the 3^{rd} wave.

Chapter 3

3.0 MATERIALS AND METHODS

Chapter 3

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3.1 INTRODUCTION

The main objective in this study was to produce viable goat offspring through embryo transfer (ET) technique. Experiments on embryo flushing following superovulation and subsequent transfer of embryos were carried out at 2 different laboratories: a) Animal Biotechnology-Embryo Laboratory (ABEL), Institute of Biological Sciences, Faculty of Science, University of Malaya, Malaysia and b) Yunnan Agricultural University, Yunnan, China. In Kuala Lumpur, the donor and recipient does were managed at Institute of Biological Sciences (ISB) Farm (Figure 3.1), University of Malaya, Kuala Lumpur, while surgeries for embryo flushing and transfer were conducted twice a month involving 2 to 3 donor does and 2 to 4 recipient does per surgery session, at the Nuclear Transfer and Reprogramming Laboratory (NaTuRe), Institute of Research Management and Monitoring (IPPP), University of Malaya, Kuala Lumpur. In Kunming, surgeries were conducted four times totaling 35 donor does and 158 recipient does. Surgeries were conducted either at the Xundian Generalstud Farm in Xundian, Kunming, or Qianshun Farm in Shiling, Kunming, Yunnan (Figure 3.2). In Kunming, the medium and regent preparations were carried out in a mobile embryonic laboratory (Figures 3.3 and 3.4). This study was conducted at two locations (Malaysia and China) in order comparison can be made on the performance of the embryo transfer in goats to separate environments (both in longitude and latitude). The breeds used in this study were selected from the available breeds at the two locations. This research project was conducted from July 2009 until March 2011.

3.2 EXPERIMENTAL ANIMALS

In Kuala Lumpur, a total of 64 donor and 19 recipient does from Boer x Katjang goats (Figure 3.5) were used in this research. In Kunming, 20 donor does from Yunnan Black mountain goat (Figure 3.6), 15 donor does from Guishan Red-bone goat (Figure 3.7) and 158 of recipient does from Saanen goat (Figure 3.8) were used. Both donor and recipient does, aged from 1.5 to 5 years old, and body weight ranged between 18 to 50 kg, were chosen as experimental animals of this study. The experimental animals were managed individually in a single pen and they were fed with commercial pellets in the morning and Napier grass (Kuala Lumpur) or ryegrass (Kunming) in the afternoon as well as given water *ad libitum*. In Kuala Lumpur at University of Malaya, experimental animals were transported for surgeries from IBS farm to NaTuRe laboratory and back to ISB farm (about 1 km apart) after surgeries using a farm transport.

3.3 MATERIALS

Materials used in this project included various equipment, reagents, chemicals and media, labwares as well as disposables. Detailed description of the materials used is given in the following sections:

3.3.1 Equipment

A list of equipment used in this project with model number, manufacturer's and supplier's name is presented in Appendix Table 1.1.

The commonly used equipment include dissecting microscope, CO_2 incubator, autoclave, mobile embryonic laboratory, digital balance, ultrasound scanner, laminar flow cabinet, laparoscopic system, micropipette dispenser, oven, pH meter, stage warmer, stereomicroscope, inverted microscope, surgical set, surgical table, ultrapure purification water system and water bath.

Figure 3.1: Institute of Biological Sciences (ISB) Farm, University of Malaya, Kuala Lumpur, Malaysia.

Figure 3.2: Qianshun Farm in Shiling and Xundian Generalstud Farm in Xundian, Kunming, China.

Figure 3.3: Mobile embryonic laboratory (outside view).

Figure 3.4: Mobile embryonic laboratory (inside view).

Figure 3.5: A typical cross breed goat used as donor and recipient does in Kuala Lumpur (Boer x Katjang).

Figure 3.6: The donor goat in Kunming (Yunnan Black mountain goat does).

Figure 3.7: The donor goat in Kunming (Guishan Red-bone goat does).

Figure 3.8: The recipient goat in Kunming (Saanen does).

3.3.2 Reagents, Chemicals and Media

Analytical grade reagents and laboratory chemicals were used in the preparation of all solutions and media. Reagents and chemicals used, otherwise stated, were purchased mainly from Sigma-Aldrich (USA) and Bioniche (Canada). A detailed list of the reagents and chemicals and media with catalogue numbers, manufacturer and supplier names is presented in Appendix Table 1.2.

3.3.3 Labwares and Disposables

A list of labwares and disposables with manufacturer names used in the study is tabulated in Appendix Table 1.3.

3.4 METHODS

3.4.1 Preparation of Stock Solutions and Media

All culture media and stock solutions used throughout the present study were prepared by the reliable and approved grades of reagents and chemicals. Ultrapure water (Millipore USA) to prepare the culture media was used throughout the experimental periods.

Preparation of different culture media requires accurate measurements as well as time consuming. Therefore, it is essential and convenient to prepare media from a series of stock solution, and frequently fresh oocyte or embryo culture media were prepared weekly or fortnightly (Nagy *et al*., 2003). All the stock solutions and media were prepared under a laminar flow work station (Gelman Sciences, Austrialia). Using syringe filter (0.22 μm pore size, Millipore USA) for prepared fundamental stock solution, aliquot in microcentrifuge tube and stored in the refrigerator (2-8°C) or freezer (-20°C) as appropriate. For all the media, the pH was adjusted to 7.2 to 7.5 (Hanna Instruments, Singapore).

3.4.1.1 Preparation heparinised saline solution

For the preparation of haparinised saline solution, NaCl (9 g) and heparin (0.05 g) were weighed by digital balance and dissolved in Milli-Q water (1000 ml) and stirred gently to mix the solution (Table 3.1). This saline solution was sterilised by autoclaving and could be kept for 3 months in the refrigerator (4°C) for use.

Table 3.1: Composition of heparinised saline solution with a shelf life of 3 months (stored at 4°C)

Chemical (catalogue number)	Concentration	Quantity/1000 ml
NaCl (S5886)	0.90% (w/v)	9.000 g
Heparin (H0777)	0.05 mg/ml	0.025 g

3.4.1.2 Preparation of flushing medium

Flushing medium (500 ml) was prepared within 12 hours before embryo retrieval form the uterine horns. The flushing medium consisted of Dulbecco phosphate-buffered saline® (DPBS) supplemented with gentamicin sulphate salt (30 μg/ml) and heparin (52 IU/100 ml) as shown in Table 3.2. The resulting medium was filter-sterilised using syringe filter (0.22 μm pore size), aliquot into Terumo® luer slip syringe (50 ml) and maintained at heating stage (Tokai Hit Japan) with 38.5°C prior to embryo retrieval.

Table 3.2: Composition of flushing medium (500 ml)

Chemical components (catalogue number)	Final concentration	Quantity/500 ml
Dulbecco phosphate-buffered saline(DPBS) tablets (P4417)	1 tablet/100 ml	DPBS (5 tablets) were dissoloved in Milli-Q water (500 ml), sterilised by autoclaving.
Gentamicin sulphate salt (G3632)	30 μg/ml	Gentamicin sulphate salt (15 mg) was dissolved in DPBS solution (500 ml) prior to use.
Heparin (H0777)	52 IU/100 ml	Heparin (260 IU) was dissolved in DPBS solution (500 ml) prior to use.

3.4.1.3 Preparation of modified SOF (mSOF) medium

In this research, mSOF medium was used as the base medium for *in-vitro* culture (IVC). For convenience, a concentrated stock of the components of mSOF at 10x concentration designated as mSOF stock medium was first prepared followed by the mSOF working solution (1x concentration).

3.4.1.3(a) Preparation of mSOF stock medium

The mSOF stock medium contain NaCl (3.15 g), KCl (266.90 mg), KH_2PO_4 (80.90 mg), $CaCl_2.2H_2O$ (125.70 mg), $MgCl_2.\ 6H_2O$ (49.80 mg) and phenol red (25 µl). The concentration and volume/weight of each of the components of the medium is presented in Table 3.3.

Table 3.3: Composition of mSOF stock medium (10x) with a shelf life of 3 months (stored at 4°C)

Chemical (catalogue number)	Concentration (10x)	Quantity/50 ml
NaCl (S5886)	107.70 mM	3.1500 g
KCl (P5404)	7.16 mM	0.2669 g
KH_2PO_4 (Prod29608)	1.19 mM	0.8090 g
$CaCl_2.2H_2O$ (C7902)	1.71 mM	0.1257 g
$MgCl_2.\ 6H_2O$ (M2393)	0.49 mM	0.0498 g
Phenol red (15100-43)	1.00 µl/ml	0.0250 ml
Milli-Q water	-	49.9800 ml

3.4.1.3(b) Preparation of mSOF working solution

The mSOF working solution was prepared with mSOF stock medium at 1x concentration as presented in Table 3.4.

Table 3.4: Composition of mSOF working solution (1x) with a shelf life of 2 weeks (stored at 4°C)

Component (catalogue number)	Concentration (1x)	Quantity/100 ml
mSOF stock solution	1x	10.0000 ml
$NaHCO_3$()	25.07 mM	25.0700ml
Sodium pyruvate (P3662)	0.30 mM	3.6000 g
Sodium DL-lactate (60% syrup) (L4263)	3.30 mM	0.0548 g
Gentamicin sulfate salt (G3632)	50.00 µl/m	0.0050 g
L-glutamine (M3126)	1.00 mM	0.0146 g
BME amino acids solution (B6766)	1x	2.0000 ml
MEM non-essential amino acids solution (M7145)	1x	1.0000 ml
Milli-Q water	-	61.7000 ml

3.4.2 Protocols for Donor and Recipient Does Preparation

A constant supply of goat embryo samples used throughout this research was from superovulated does, and embryo transferred to the recipient does. In the present study, two different hormone treatments on donor does were designed and carried out. For recipient does, the hormone treatment is given the same which has been described as below.

In Experiment 1 (Kuala Lumpur), all does (donors and recipients) were inserted with one CIDR® (0900 hours) for 14 days to synchronise the oestrous cycle. For donor does, they were injected with Estrumate® (125 µg) on day 13 (0900 hours) and PMSG® (1200 IU) 1 day (0900 hours) after the Estrumate injection. CIDR was withdrawn on day 15 (1800 hours). On days 16 and 17, hCG (500 IU) was injected each day (0900 hours) and check for oestrus for 3 sessions per day: morning (0900 to 1000 hours), afternoon (1600 to 1700 hours) and night (2000 to 2100 hours) by the presence of a

buck per donor during the oestrus period. Natural mating or AI was carried out during the oestrus observation period by allowing the fertile buck to mount the doe. At the same time, new buck was introduced to the doe for different oestrus observation periods. This process was carried out until the absence of oestrus behaviour. As for the recipient does, they were injected with 300 IU PMSG (i.m; 0900 hours) on the day 14 and CIDR was withdrawn on day 14 (1800 hours). The embryo flushing and transfer were performed on day 21 (7 days after CIDR withdrawal) (Figures 3.9 and 3.10).

In Experiment 2 (Kuala Lumpur and Kunming), all animals (donor and recipient does) were inserted with one CIDR for 17 days to synchronise the oestrous cycle. The donors were injected with FSH-V® (total: 160 mg; i.m.) from days 14 to 17, each day twice consecutively (20 mg; i.m.; 0900 and 2100 hours). The recipient was injected with 300 IU PMSG (i.m; 0900 hours) in the day 17 and in the same day CIDR was withdrawn (1800 hours). Oestrus was checked with the same method like in Experiment 1 from day 18 until no oestrus was observed. The embryo flushing and transfer were carried out on day 24 (7 days after take out the CIDR) (Figures 3.11 and 3.12).

In Experiment 3 (Kunming), all does (donor and recipient does) were treated according to the protocols as described in Experiment 2.

3.4.3 Insertion of the CIDR

The controlled internal drug release devices (CIDR, 0.3 g progesterone) used for goats oestrus synchronisation (Figures 3.13 and 3.14). The CIDR was inserted to the vagina to a depth of 4 to 5 cm by CIDR applicator with jelly (K-Y Lubricating Jelly®). Before insertion, the doe's vagina was wiped with 70% alcohol, after insertion, the applicator was cleaned with 70% alcohol, before being used for the next doe. For each time of the experiment, the doe was checked every day for the presence of the CIDR and not

dropped from the vagina. If the CIDR dropped during the period of synchronisation, a new CIDR was replaced immediately.

3.4.4 Take out of CIDR

The CIDR was withdrawn from the doe on days 14 or 17 after insertion in Experiment 1 and Experiment 2 and 3 does respectively, by gently and fast taking out the string.

3.4.5 Detection of Oestrus

After CIDR out, the doe in oestrus behaviour was checked by a buck. For donor doe, oestrus checking was done by using a buck 3 sessions daily: namely in the morning (0900 to 1000 hours), afternoon (1600 to 1700 hours) and night (2000 to 2100 hours) after CIDR withdrawal. If the doe was showing oestrus signs, she would allow the buck to mount her. Then the buck with the doe in oestrus was placed in a single pen and observed for the oestrus activities (mounting and mating) for 1 hour during each observation session. To ensure maximum fertility and sperm quality, a different buck was introduced to the doe for each observation session until the end of oestrus period. For the recipient doe, oestrus checking by buck was carried out simultaneously with the donor doe, records of the oestrus activities were made for both the donor and recipient does.

3.4.6 Artificial Insemination (AI)

Artificial insemination was carried out for all the oestrus donor does in Kunming and some of the predetermined selected oestrus donor does in Kuala Lumpur (Figure 3.15). AI was carried out using fresh sperm collected from fertile bucks (Kunming and Kuala Lumpur), except in some cases, frozen sperm were used in Kuala Lumpur.

3.4.7 Ultrasound Scanning Procedure

After took out of CIDR and transferred the embryos, both the donor and recipient does were checked for follicular growth, CL and pregnancy using ultrasound scanner (Figure 3.16). With transrectal approach, faeces were cleared from the rectum. The 7.5 MHz transducer was attached to the tip of a rigid extension rod. The tip of the transducer was lubricated with carboxyethylcellulose contact gel and the transducer was gently inserted until the urinary bladder was identifiable. The probe was moved gently forwards and backwards and rotated 90° clockwise and 180° counter-clockwise. Images of Graafian follicles, corpus luteum, embryonic sac and foetus were identified.

3.4.8 Preparation for Surgery

To ensure cleanliness and success of surgery, the surgeon and surgery team were always paid attention to sterility and proper attires which include: cut the nail, cleaned the hands using disinfectant, wore clean surgery gown, surgery hat, glove and mask. A list of surgical instruments used in the study is presented in Figure 3.17.

3.4.9 Preparation of Donor Does for Surgery

The does should be off-feed and water 12 hours before the surgery. Before bringing to the surgery room, the does were anaesthesised using xylazine hydrochloride® (Troy Laboratories, Australia, i.m.; 0.22 mg/kg body weight) followed by ketamine hydrochloride® (Troy Laboratories, Australia, i.m., 11 mg/kg body weight). After the does were anaesthesised, they were placed on surgery table and the legs were tied to reduce movement (Figure 3.18).

3.4.10 Disinfection of Surgical Position

Using the electric clippers (Kunming) or razor blade (Kuala Lumpur), the hair was shaved at the lower abdomen near the udder. The area size about 15 square centimeter, then use the sterile gauze clean the surgical area by disinfectant fluid and 2 to 4% tincture iodine. Finally the surgical drape was placed and clipped properly and tightly over the place of surgery to be made (Figure 3.19).

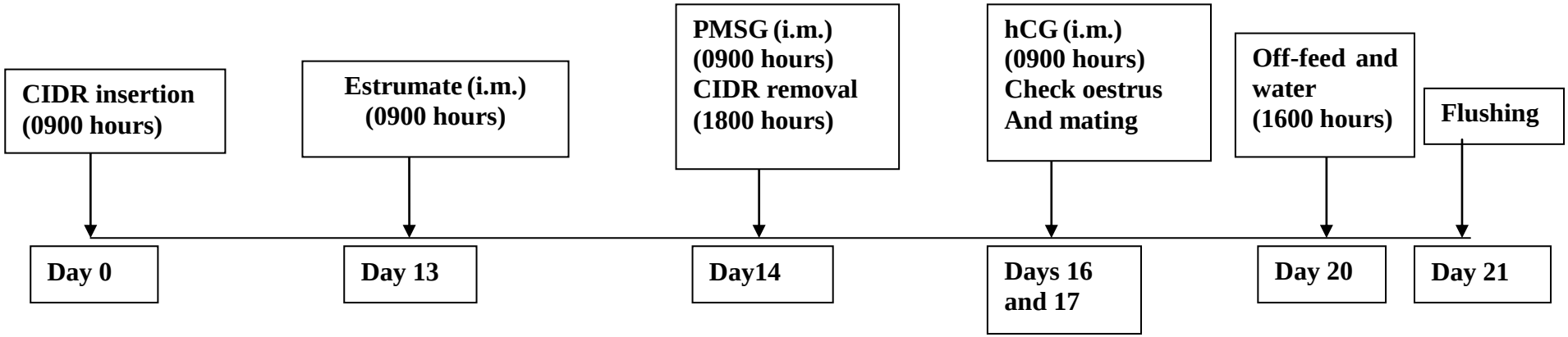


Figure 3.9: Oestrus synchronisation and superovulation procedures for donors inserted with CIDR for 14 days and injected with PMSG (in Kuala Lumpur).

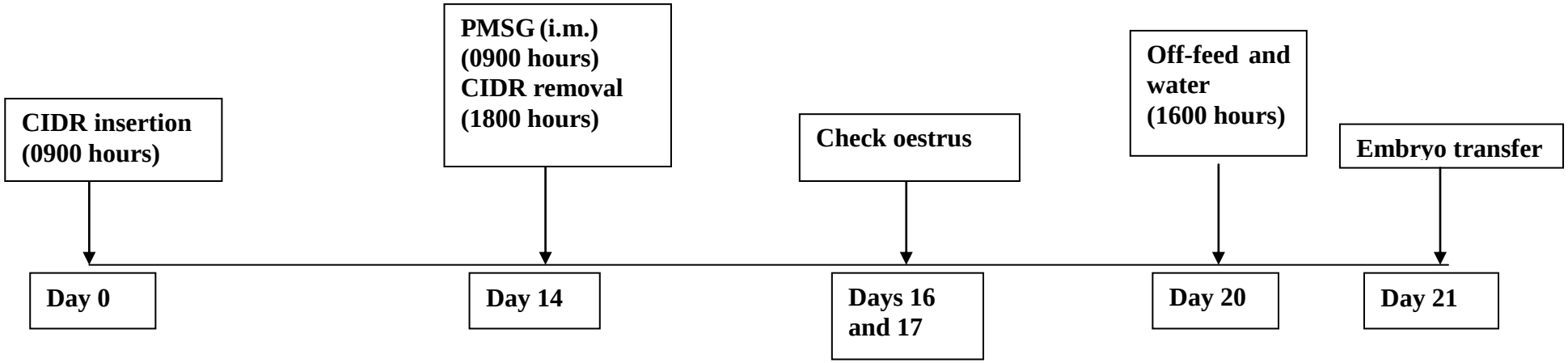


Figure 3.10: Oestrus synchronisation procedures for recipients inserted with CIDR for 14 days and injected with PMSG (in Kuala Lumpur).

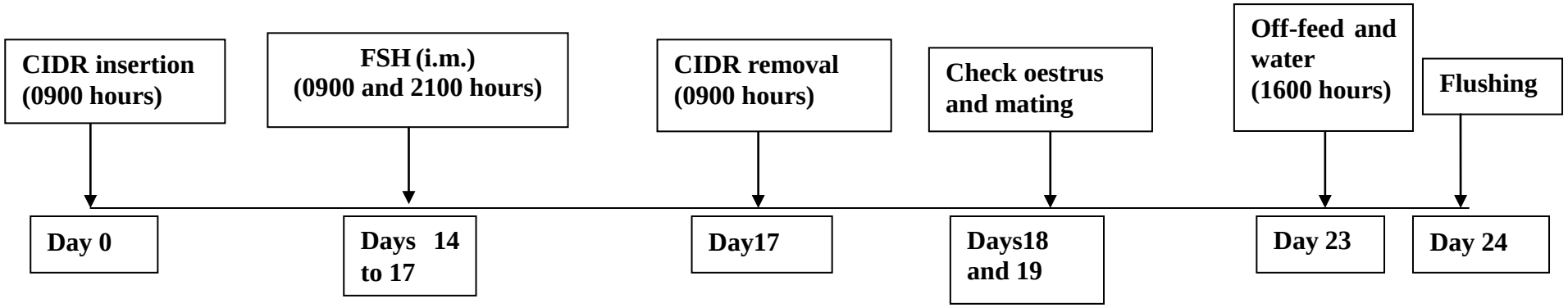


Figure 3.11: Oestrus synchronisation and superovulation procedures for donors inserted with CIDR for 17 days and injected with FSH-V (in both Kuala Lumpur and Kunming).

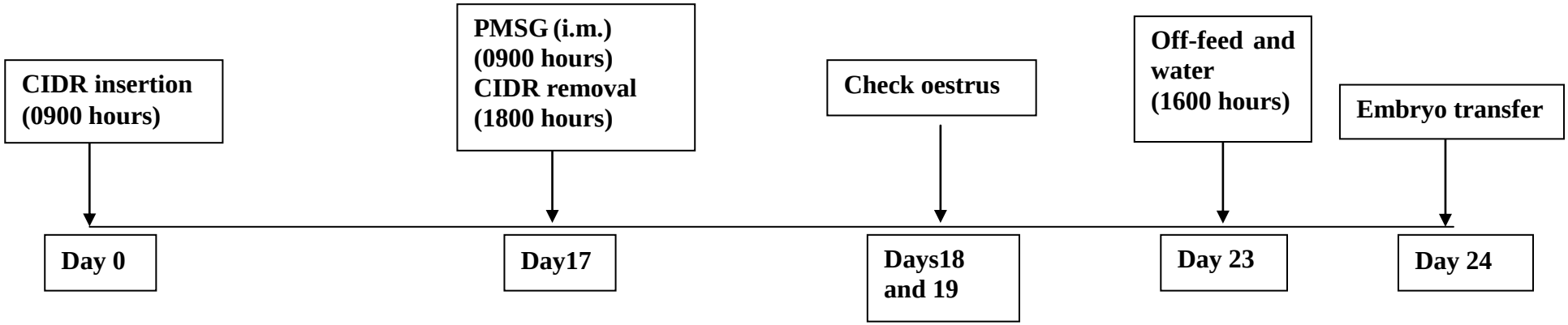


Figure 3.12: Oestrus synchronisation and superovulation procedures for recipients inserted with CIDR for 17 days and injected with PMSG (in both Kuala Lumpur and Kunming).

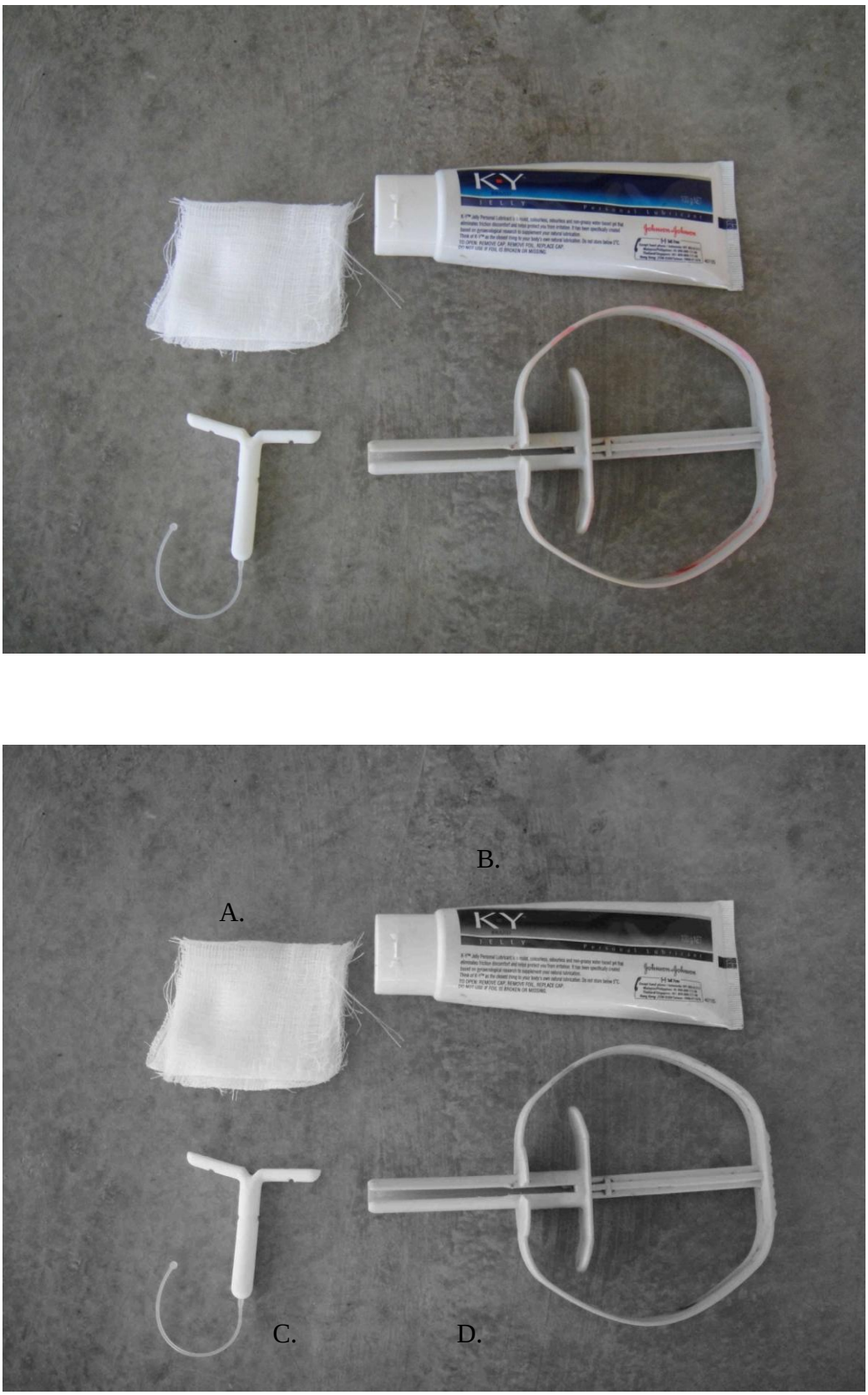
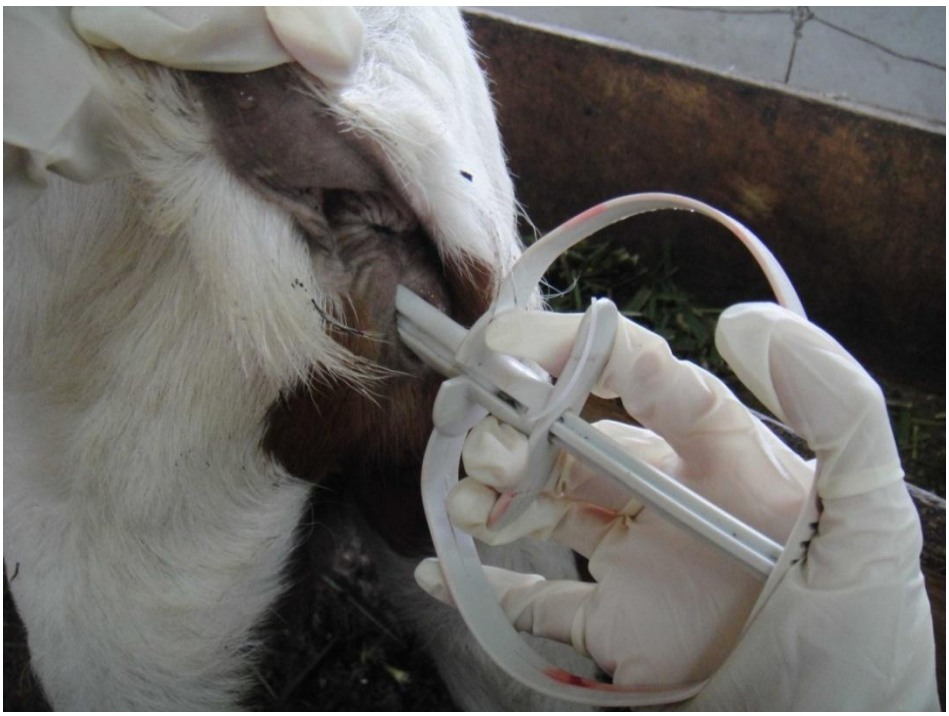


Figure 3.13: Items used for the introduction of insertion of CIDR: (A) Aseptic gauze, (B) K-Y Lubricating Jelly, (C) Controlled Intravaginal Durg Release device (CIDR®), (D) CIDR applicator.



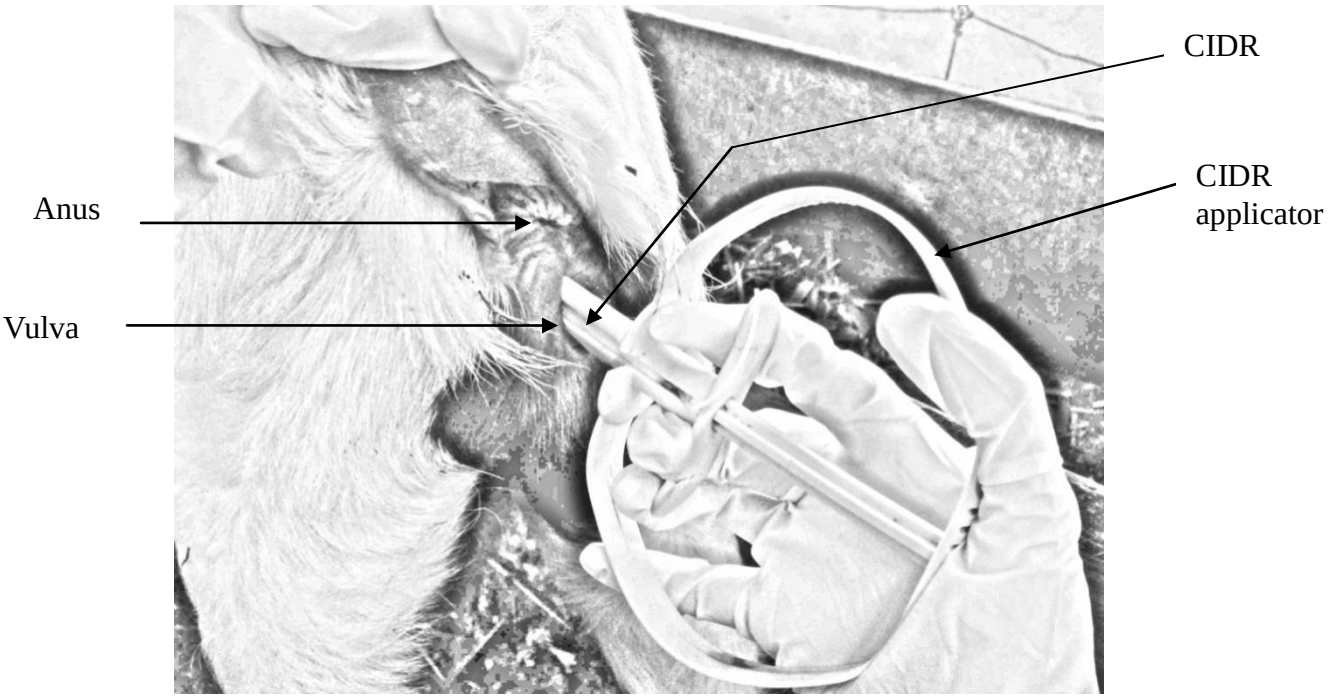
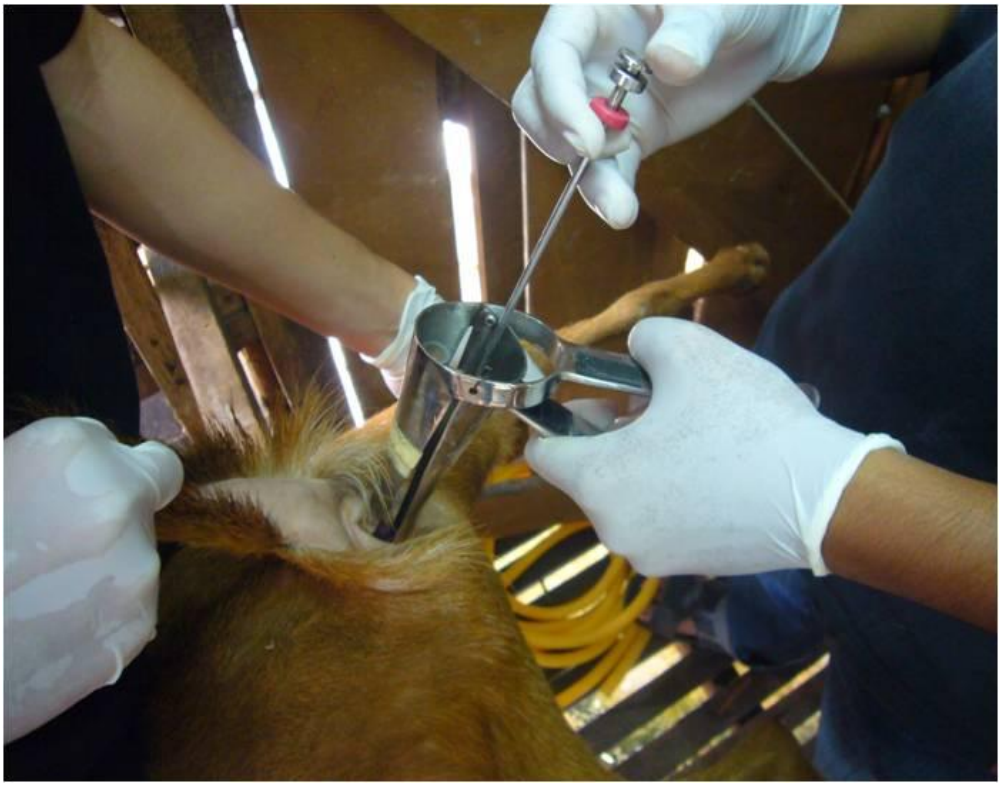


Figure 3.14: Insertion of the CIDR into the vagina of the doe.



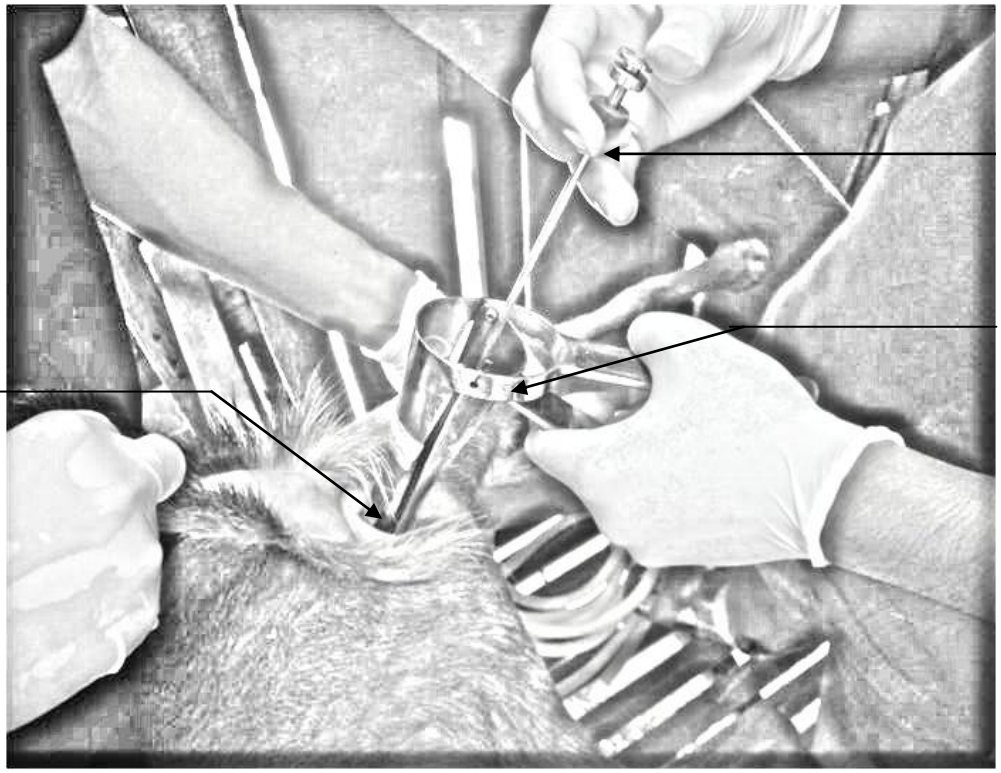


Figure 3.15: Artificial insemination for the donor doe.

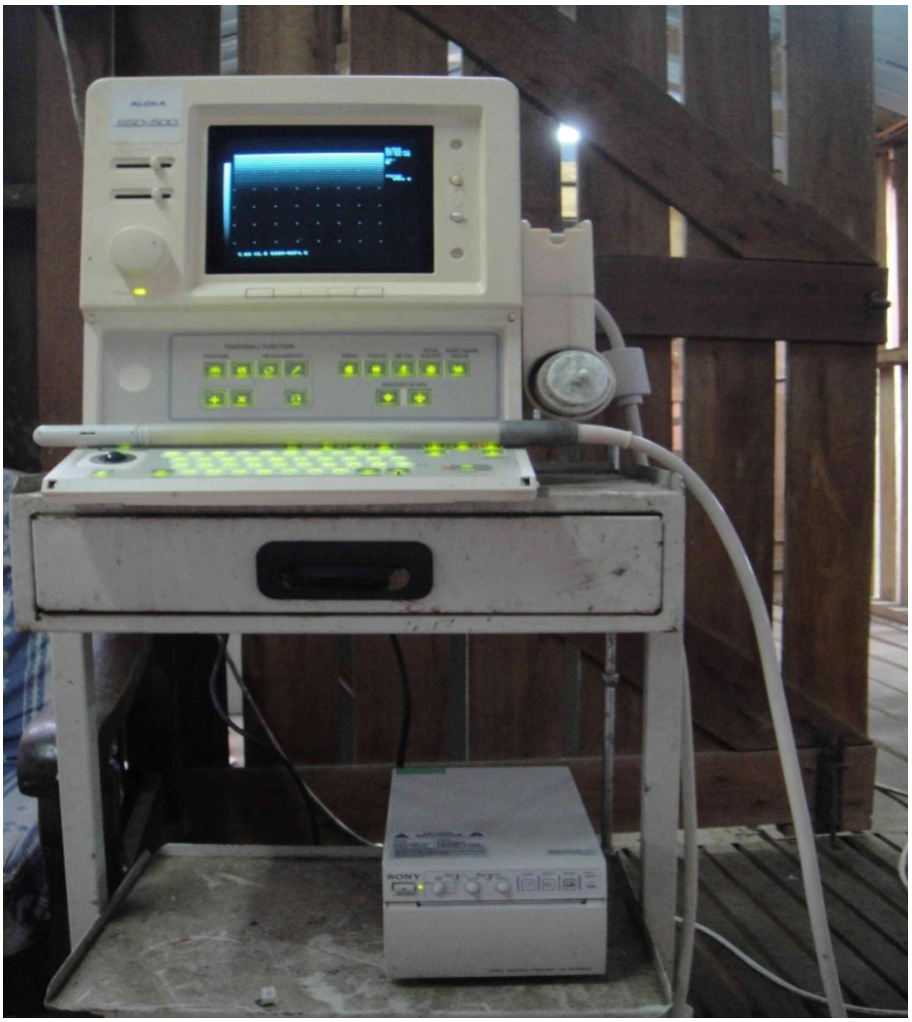


Figure 3.16: Ultrasound machine ALOKA SSD500. This ultrasound machine Comprises of 3 major parts; monitor, probes and printer. ALOKA SSD500 can be used for B-mode and M-mode ultrasound diagnosis.

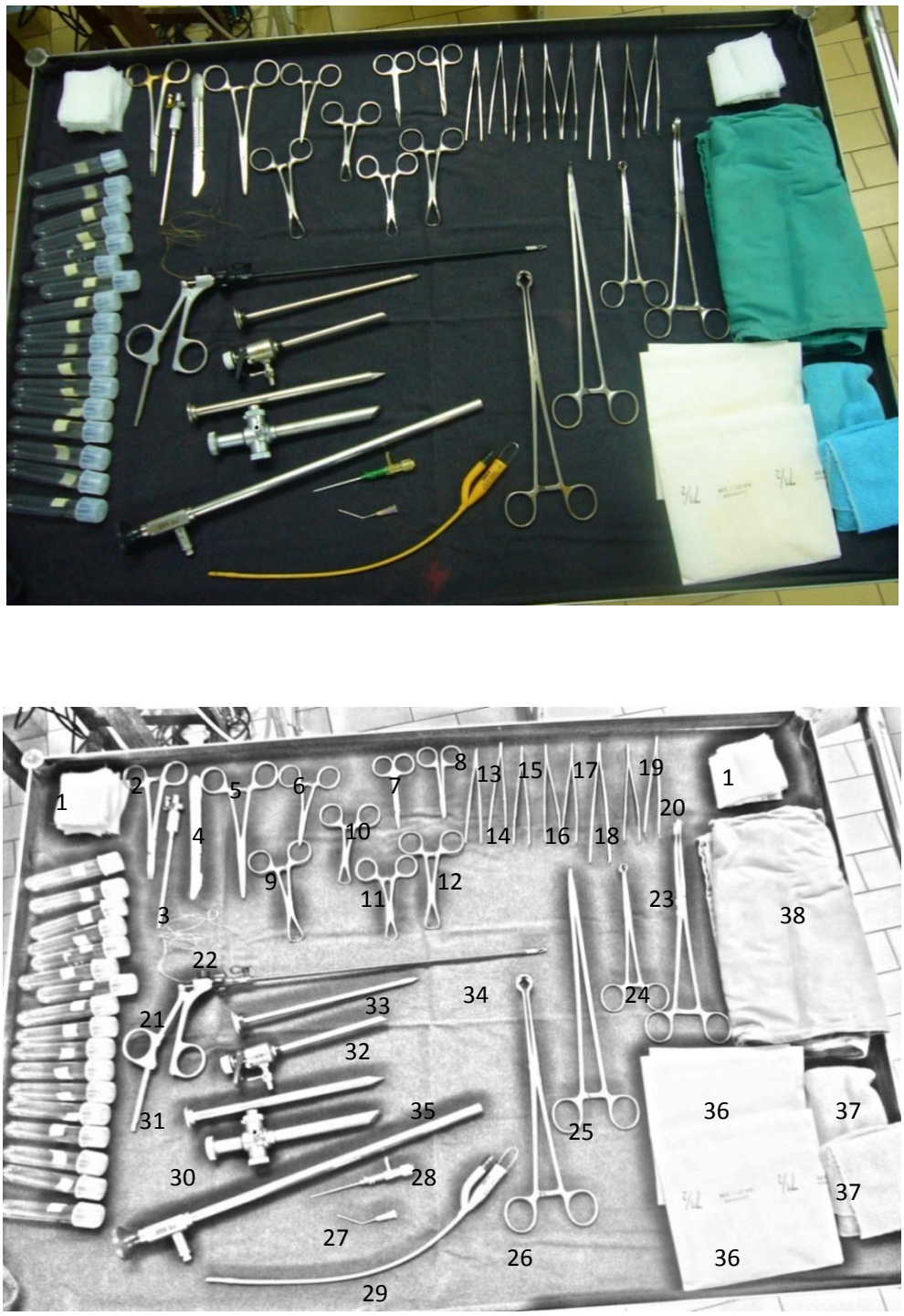


Figure 3.17: Setting up of surgical instruments on surgical trolley. The items included (1) sterile gauze, (2, 7, 8) different types of surgical scissors, 3) Veress needle,(4) scalpel with surgical blade, (5, 6, 9-12, 23-26) different types of haemostatic forceps, (13-20) different types of forceps, (21) collecting tubes,(22) suture, (27) needle, (28) Teflon I.V. Catheter,(29) Foley catheter, (30, 32) cannula, (31, 33) trocar, (34) atraumatic grasper,(35) light probe for endoscope, (36) sterile gloves, (37) sterile hand towel and (38) drape for animal.

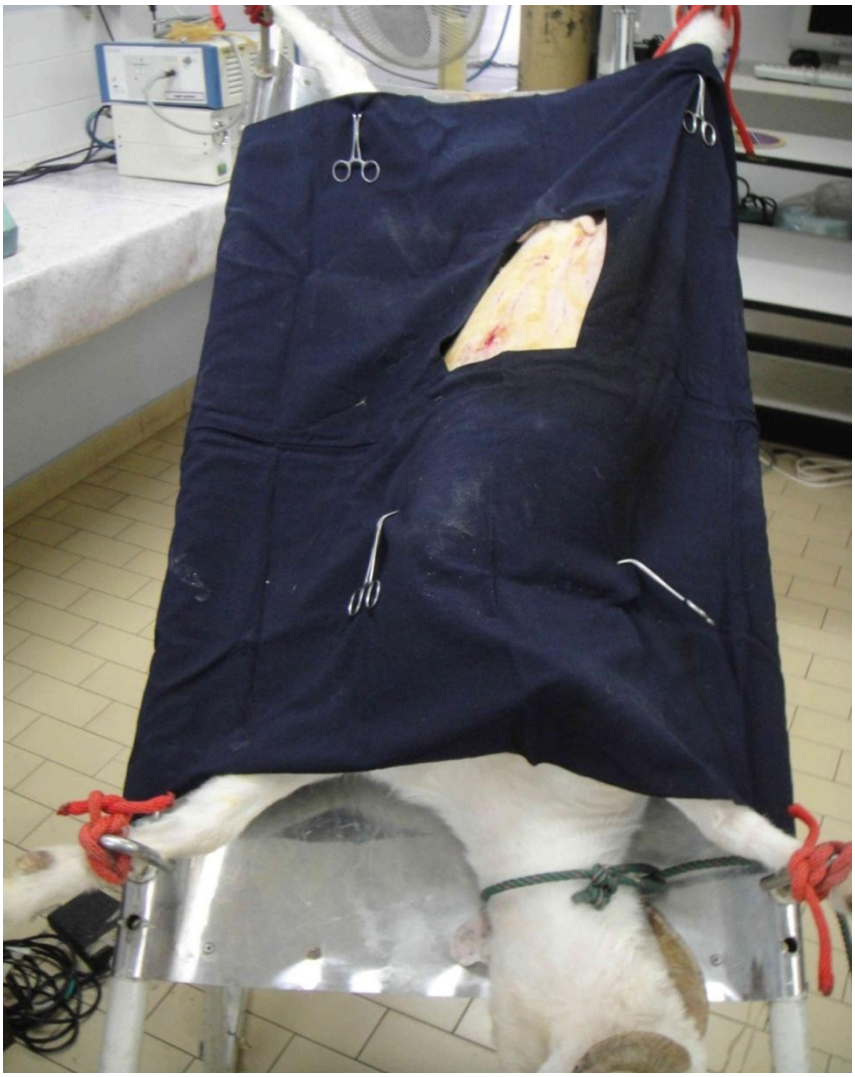


Figure 3.18: Position of the doe with its leg securely tied on the surgical table.

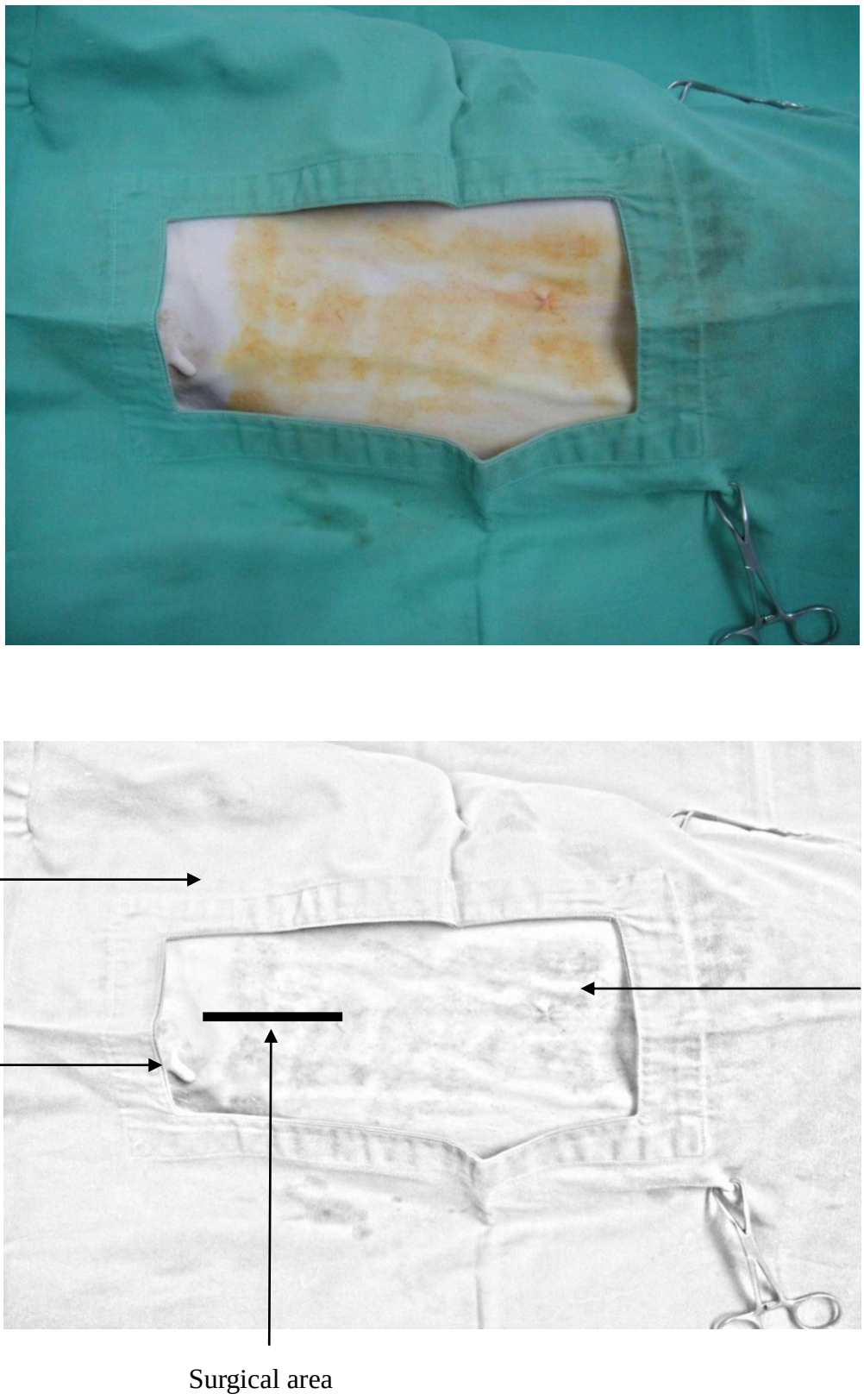


Figure 3.19: The location of surgical area on the doe abdomen.

3.4.11 Surgery

The embryo flushing and embryo transfer surgery were performed in the surgery room by a team of members consist of the surgeon, assistant surgeon and at least two research assistants (Figures 3.20 and 3.21).

The surgery was commenced by using a scalpel to make a mid-ventral incision through the skin and muscle and at the same time, precautions were made not to cut the main blood vessel, nerve, and previous surgical scar (if any). If the main blood vessel was cut accidently, it should be stitched immediately to prevent major bleeding.

a) **Cut the skin:** using the left hand index finger and first finger the skin was shoved off, and by using right hand holding the scalpel, the skin was cut from beginning to end without stopping and the depth of the incision was kept relatively constant.

b) **Cut the hypodermis:** similarly, using the scalpel small hole was cut and then follow by a snip by using a surgical scissors.

c) **Cut the muscle:** tracking the direction of muscle fibre, a small hole was stabbed by a haemostat, and then the muscle was peeled following the direction of fibre by using a finger.

d) **Cut the peritoneum:** using the tweezers, the peritoneum was lifted, and then a small hole was cut, and precaution was made to avoid cutting the viscera. Following that, using the other index finger, the peritoneum was open by using the surgical scissors.

3.4.12 Flushing the Embryos

The index finger and middle finger were placed in the enterocoelia through the cut; and the uterine horn was searched in pelvis cavity. Using two fingers, the uterine horn was held and pulled gently up to expose the oviduct and the uterus as well as the ovary for preliminary evaluation of the ovary status before deciding to precede the flushing or embryo transfer. Both for the donor and recipient does, number of corpus luteum (CL) was recorded (Figure 3.22). If no corpus luteum was observed, the flushing of the donor or the embryo transfer of the recipient was cancelled and the respective doe was sutured. If there were corpus luteum, the uterus was pulled out to expose it to facilitate flushing or embryo transfer procedure.

For the flushing, using the tweezers, a small hole was punctured with a hole diameter fit to insert a two-way size 8 Foley Catheter to a depth of the puncture to a depth of 2 to 3 cm (Figure 3.23). The balloon of the catheter was sufficiently inflated (3 to 5 ml) to completely obstruct the lumen at the uterine horn. A Teflon intravenous (I.V.) catheter placement unit fixed with a 20 gauge needle was introduced. Then, the I.V. catheter needle was removed. The flushing was carried out by pushing slowly the medium through a 20 ml sterile disposable syringe fixed to the end of the Teflon catheter (Figure 3.24). The embryos were expected be collected at the other end of catheter by using 5 ml collecting tube after which the tube was replaced each time until the flushing procedure was completed. The uterine horn was gently massaged and the fluid contents of the uterine horn were collected through the free end of the Foley Catheter. The total volume of flushing medium used was about 50 to 80 ml. The embryos in the collecting tubes were searched and recorded using a stereo-microscope. Subsequently, the same procedure was used for the flushing of the other uterine horn.

3.4.13 Post-operative Management for Donor Does

After flushing, the uteri were filled with saline (200 ml) and the blood clot (if any) was taken out. The uteri were taken back into the enterocolia, the peritoneum, muscle and skin were sutured in proper sequence. After that, iodine and alcohol were applied.

3.4.14 Examination of Embryos

The flushing medium containing embryos was searched under a portable inverted stereomicroscope as soon as possible. The recovered embryos were maintained on a heated plate (37^{o}C) with the embryo holding solution (EMCARE, USA) until the transferring of embryos few minutes later. If the embryo transfer was carried out more than 2 hours later, the embryos would be transferred to the IVC medium and incubated in the 5 % CO_2 incubator.

After searching embryos, the embryo cell stage and grading were recorded in accordance with following procedures:

The stages of embryos are as follows:

a) *Early stage embryos*: the embryos were collected on days 2 to 3 after oestrus (fertilised) by oviduct flushing method. The embryos were from 2 to 16 cells stages and the blastomeres could be seen clearly as well as the perivitelline cavity was relatively big.

b) *Early morula*: the embryos were collected on days 5 to 6 after oestrus (fertilised). Sometime, it could be confused with the blastomeres which only could be observed as globular inner cell mass that occupied most of the perivitelline cavity.

c) *Compact morula*: the embryos were collected on days 6 to 7 after oestrus (fertilised), with inner cell mass reduced in diameter and occupied about 60 to 70% of perivitelline cavity.

d) *Early blastocyst*: the embryos were collected on days 7 to 8 after oestrus (fertilised), and appeared as cavities from a apart of the inner cell mass. Inner cell mass reduced size and occupied about 70-80% of perivitelline cavity.

e) *Blastocyst*: the embryos were collected on days 7 to 8 after oestrus (fertilised), in which the boundaries between the inner cell mass and trophectoderm were cleared. The cavity was clearly seen.

f) *Expended blasyocyst*: the embryos were collected on days 8 to 9 after oestrus (fertilised), and the size of cavity increased 1.2 to 1.5 times, with no space between oolemma and zona pellucida. Zona pellucida reduced in size to a third of the normal thickness.

g) *Hatching blasyocyst*: the embryos were collected on days 9 to 11 after oestrus (fertilised). Cavity continue to expand, resulting in the cracking of the zona pellucida, and finally the embryos were hatched out of the zona pellucida

The grades of embryo can be classified as follows:

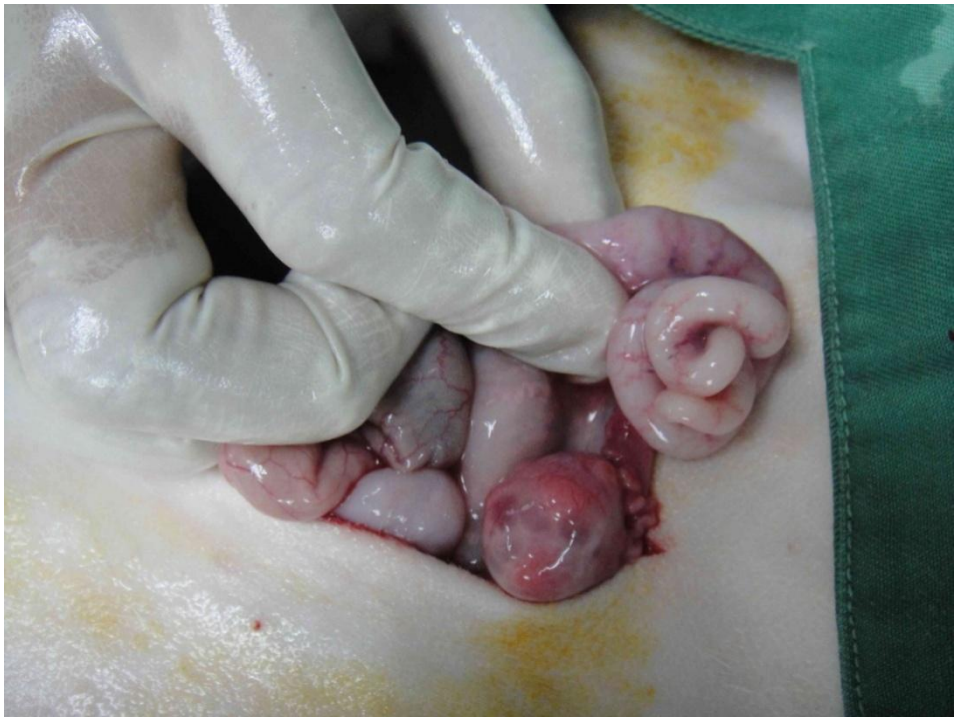
a) *Grade A*: embryo form complete, clear-cut, global, blastomere size uniform, compact structure, no adherent vacuole.

b) *Grade B*: embryo form clear-cut, good tone and cell density, a few adherent vacuoles, degeneration cell about 10 to 30%.

c) *Grade C*: embryo contour no clear, dark tone and loose structure, a lot of dissociative vacuoles, degeneration cell about 30 to 50%.

Figure 3.20: Performance of goat embryo transfer surgery by the surgeon and assistant surgeon.

Figure 3.21: Observation of the goat ovaries through laparoscopic technique.



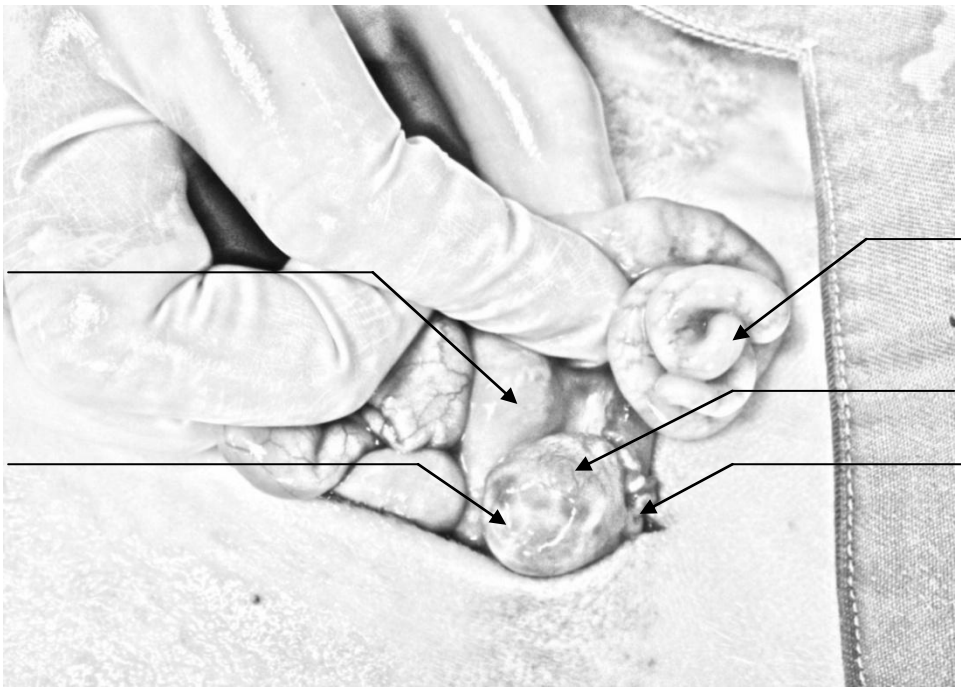
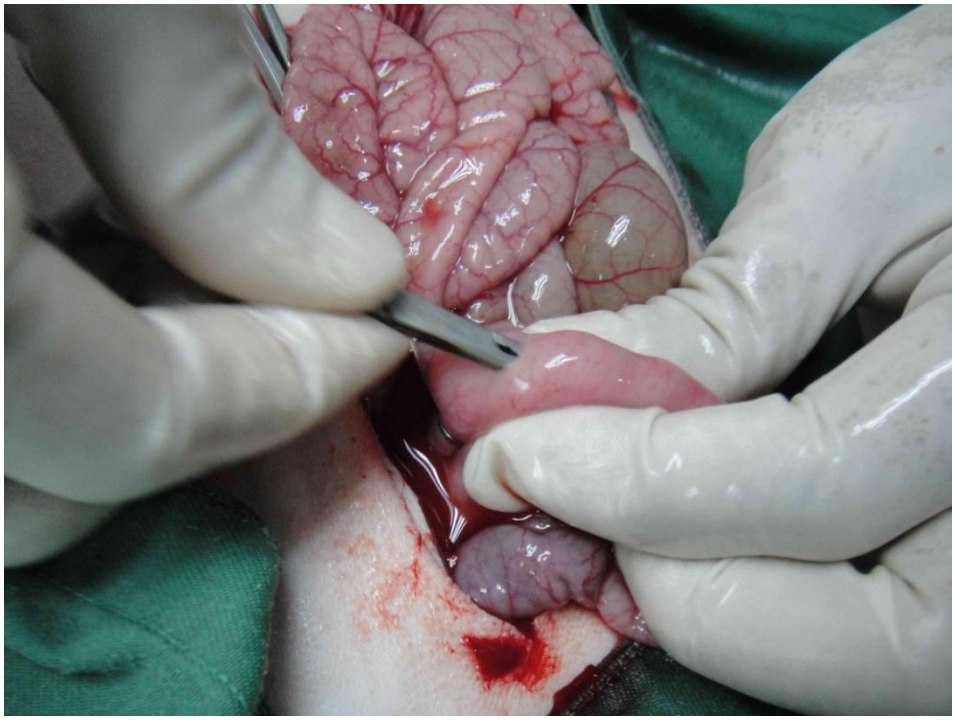


Figure 3.22: The uterine horn and ovaries were exteriorised for observation of the ovarian morphology.



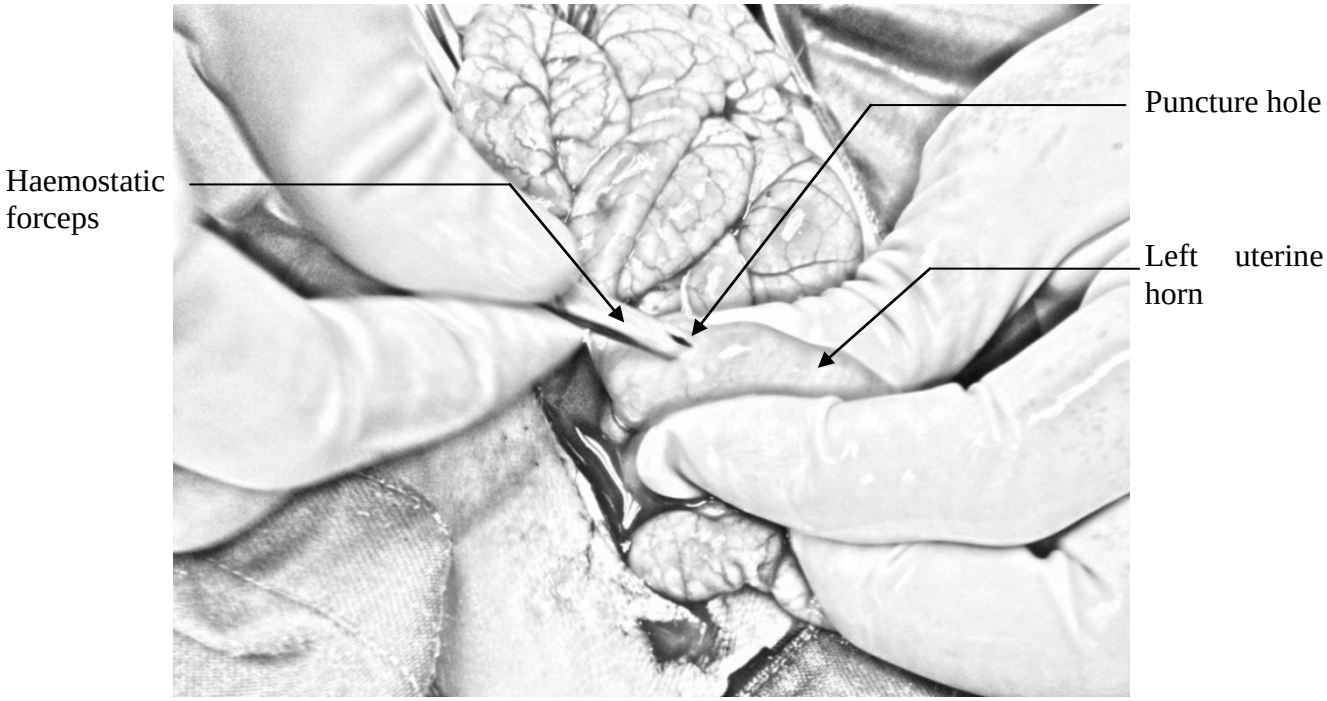
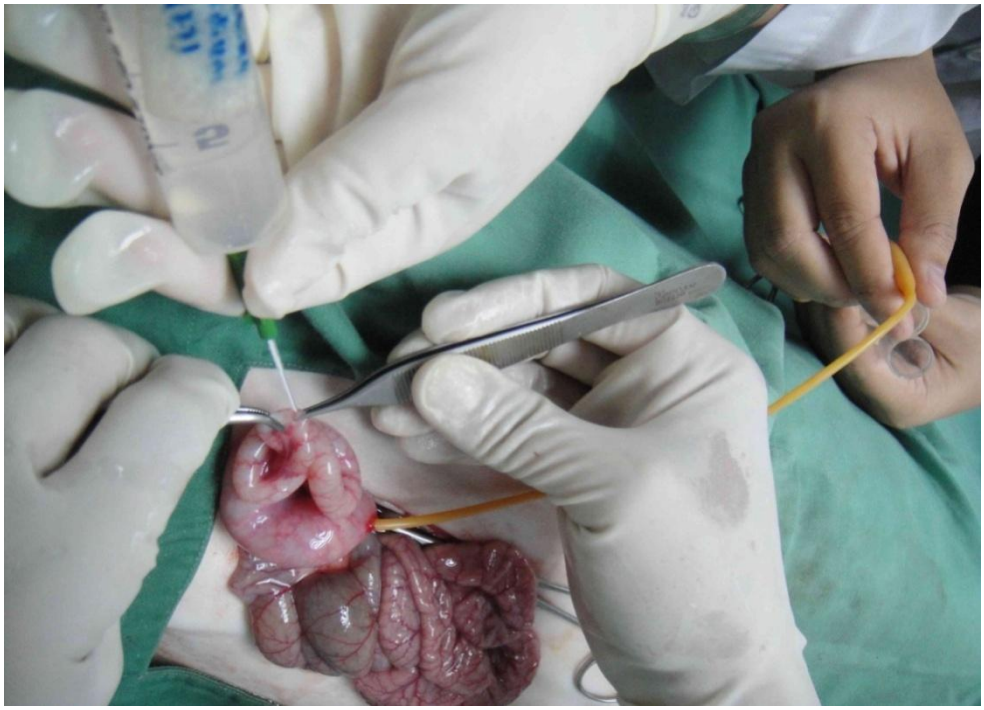


Figure 3.23: A puncture wound was made near the bifurcation of the uterine horn using a pair of haemostatic forceps.



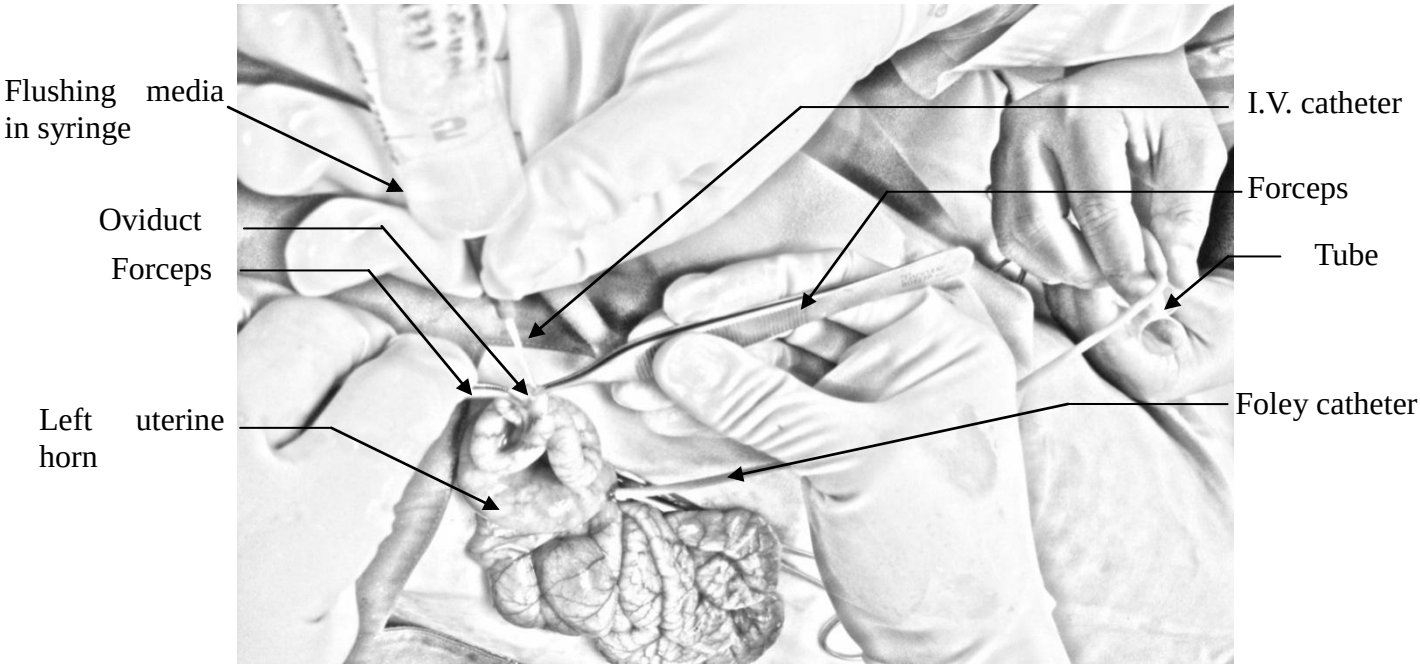


Figure 3.24: Introduction of flushing medium into the uterine horn and collection of the flushed fluid with a sterile glass tube through the Foley catheter.

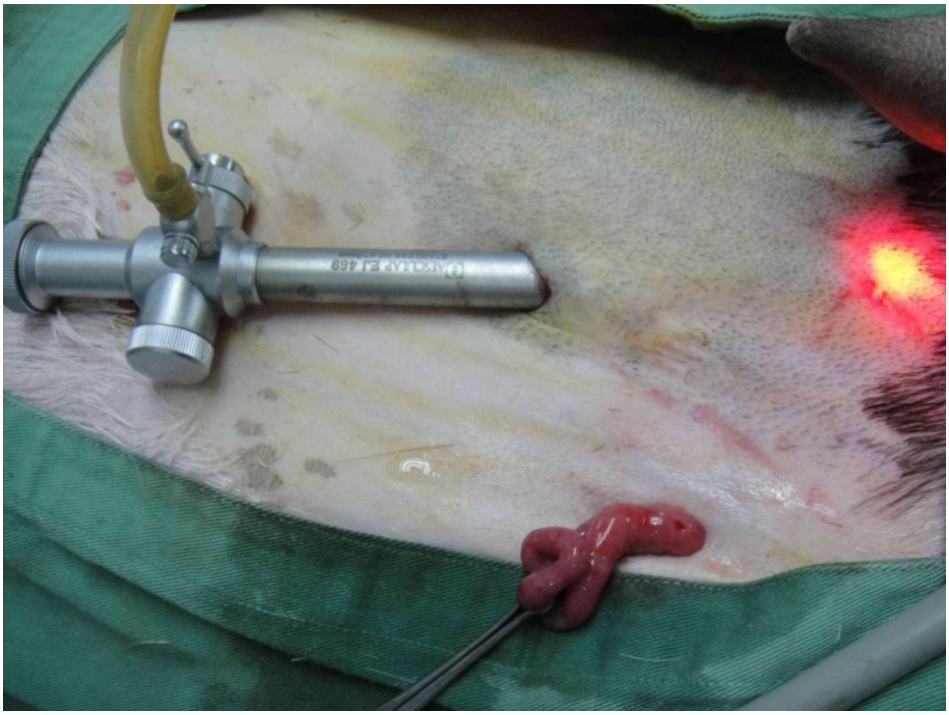
3.4.15 Embryo Transfer

The general anaesthesia procedure and preparation used on the donor animals were performed on the recipient animals for laparoscopy. When exposing the uterine horn, a 2 cm slit was made with a scalpel blade at the cannulation point and the anterior part of the uterine horn was exteriorised over the surface of abdomen. A small hole was made on the uterine horn using a 20 gauge hypodermic needle (Figure 3.25). Fresh embryos (1 to 3) were loaded into a 20 gauge Teflon I.V. catheter placement unit along with a small amount of flushing medium (0.1 to 0.2 ml) and were transferred into the lumen of the anterior part of the uterine horn through a guided hole prior-punctured using a sterile paper clip (Figure 3.26).

The procedure for the transfer of the embryos was repeated after exteriorising the opposite uterine horn.

3.4.16 Post-operative Management for Recipient Does

After embryo transfer, the uteri were placed back into the enterocolia, the peritoneum, muscle and skin were sutured in proper sequence. After that, iodine and alcohol were applied around the wound. The recipient does were transferred to single a pen and supplied with clean water and fresh grass *ad libitum*. The does were observed for 1 to 2 days for any health abnormalities. The pen was kept dry and clean to avoid the does from infection. Using ultrasound scanning, the recipient does were scanned; if pregnant, sufficient nutrition was provided to the recipient does for the foetal growth and development during pregnancy.



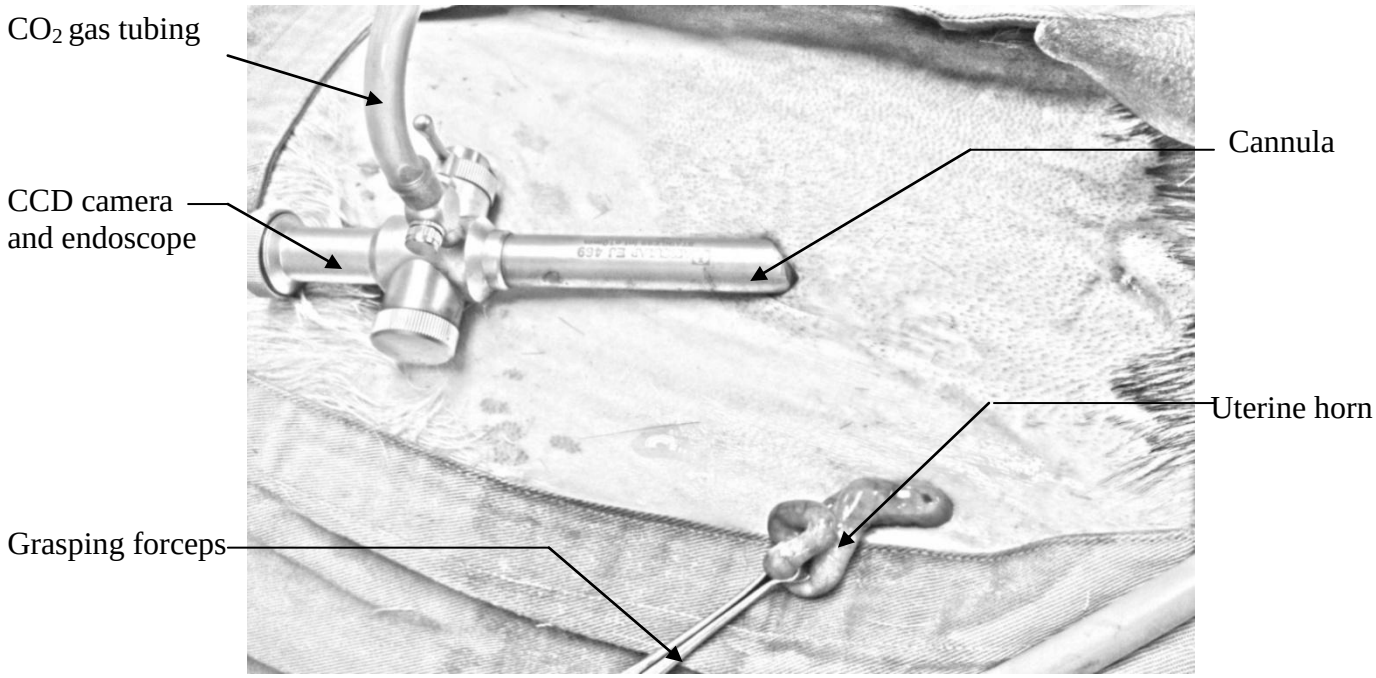
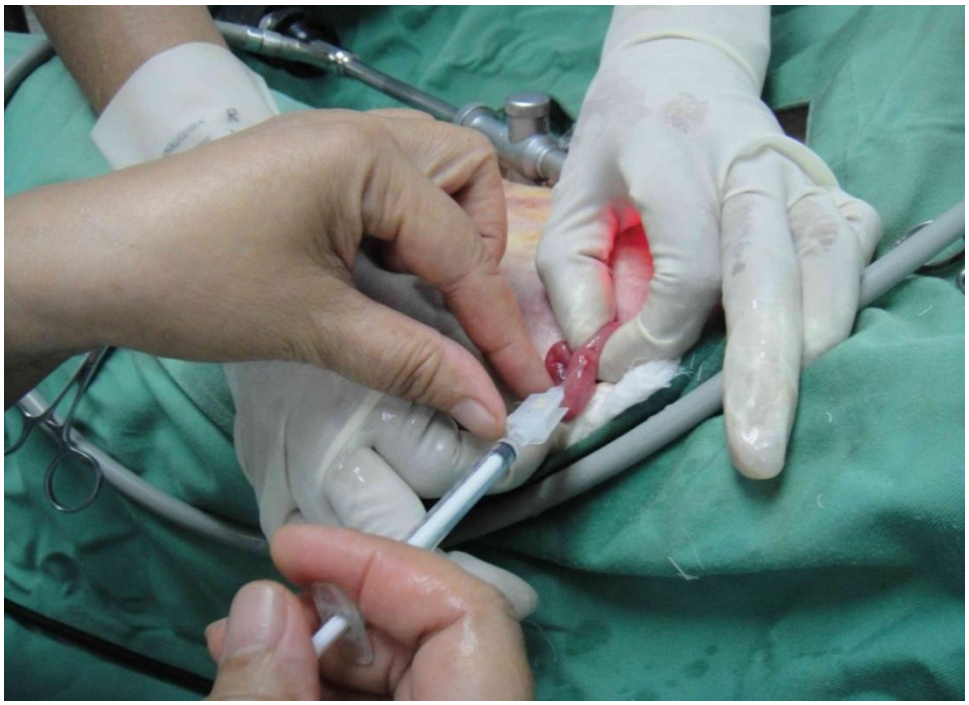


Figure 3.25: Laparoscopy in a goat. The endoscope was attached to a CCD caera for external viewing of the ovary on a video monitor. The grasping forceps was used to manipulate the uterus and ovary.



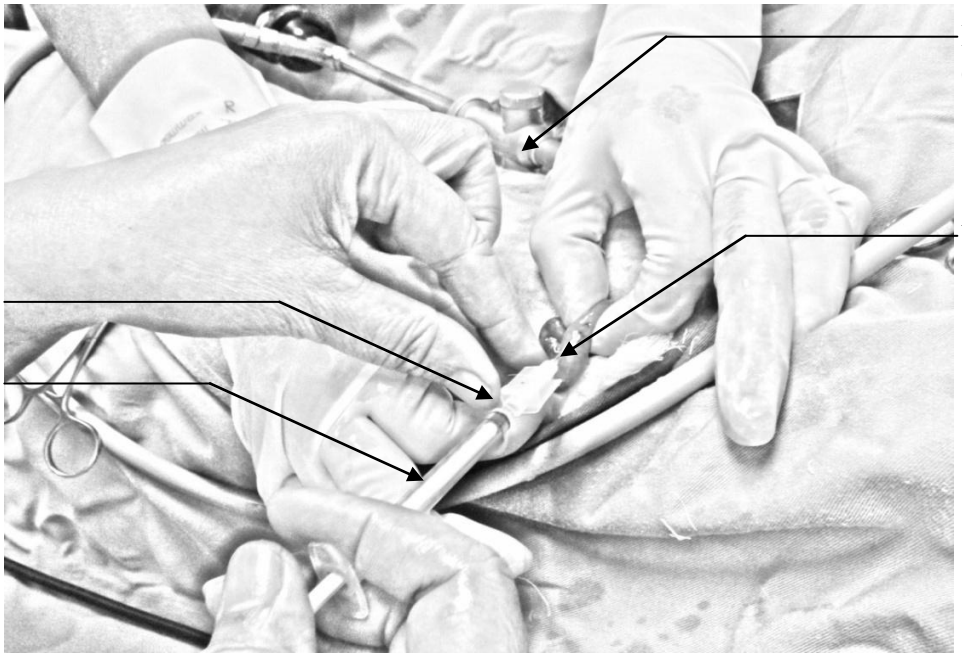


Figure 3.26: A puncture hole was made on the uterine horn using a 20 gauge hypodemic needle. Embryos along with small quantity of flushing medium were released into the lumen of the uterine horn using a 20 gauge (33 mm) Teflon I.V. catheter placement unit. Note: one of the surgeon did not wear glove, however, the surgeon washed the hands with antiseptic and thus the hands were sterilised during the surgery.

3.5 EXPERIMENTAL DESIGN

The present study was carried out to investigate the effects of different hormone treatments and locality of study during the oestrus sychronisation, superovulation on embryo recovery, pregnancy rate and kidding rate in goat.

3.5.1 Comparison of PMSG and FSH Effects in Superovulation Procedure on Embryo Recovery Rate, Pregnancy Rate and Kidding Rate in Goat After Embryos Transfer (Kuala Lumpur) (Experiment 1)

A total of 64 donors does were assigned in 2 groups: a) Group 1: CIDR (14 days) plus Estrumate (125 µg), PMSG (1200 IU) and hCG (500 IU), consisting of 47 donor does (South African Boer x Katjang) and b) Group 2: CIDR (17 days) plus FSH-V (160 mg), consisting of 17 donor does (South African Boer x Katjang). Total of 19 recipient does (South African Boer x Katjang) were treated with CIDR (14 or 17 days for Group 1 or 2, respectively) plus PMSG (300 IU), consisting of 9 recipient does for Group 1 and 10 recipient does for Group 2. After CIDR withdrawal, oestrus behaviour of donor does was checked for 3 sessions per day, and was subjected for mating with fertile bucks until a day before the surgery. For the recipient does, detection of oestrus was carried out similarly, except that no mating was allowed. Seven days after CIDR withdrawal, the donor and recipient does were prepared for embryo flushing and embryo transfer, respectively. The number of CL, number of embryos recovered, stage and grade of embryos, pregnancy rate and kidding rate were recorded. Effects of PMSG and FSH-V on the parameters measured were determined. In addition, a total of 30 superovulated donor does from Groups 1 (15 does) and 2 (15 does) were further used for the evaluation of follicular development using ultrasound scanning technique. Briefly, all the donor does were scanned using transrectal probe to scan the ovaries for follicular

structures stimulated after superovulation, that is on days 2 and 4 after oestrus. Also, number of CL was determined during embryo flushing surgery in donor does. The objective of this sub-experiment was to describe the follicular development prior to surgery for embryo recovery in superovulated donor does and related it to the actual number of embryos recovery and CL determined after surgery.

In addition, a total of 30 superovulated donor does from Groups 1 (15 does) and 2 (15 does) were further used for the evaluation of follicular development using ultrasound scanning technique. Briefly, all the donor does were scanned using transrectal probe to scan the ovaries for follicular structures stimulated after superovulation, that is on days 2 and 4 after oestrus. Also, number of CL was determined during embryo flushing surgery in donor does. The objective of this sub-experiment was to describe the follicular development prior to surgery for embryo recovery in superovulated donor does and related it to the actual number of embryos recovery and CL determined after surgery.

3.5.2 Effect of Different Localities in Embryo Transfer Programme on Superovulation Responses, Pregnancy Rate, and Kidding Rate (Kuala Lumpur and Kunming) (Experiment 2)

A total of 220 does were assigned in 2 groups: a) Group 1: CIDR (17 days) plus FSH-V (160 mg), in Kuala Lumpur, consisting of 17 donor and 10 recipient does (27 African Boer x Katjang) were synchronised using 17 days CIDR as described before. For the donor does, from day 14 to day 17 injected with FSH (20 mg) per time and twice per day, CIDR was withdrawn on day 17. Oestrus was checked for 3 sessions per day and were subjected for mating with fertile buck until the 1 day before the surgery. For the recipient does, PMSG (300 IU) was injected and take out the CIDR in the same time with the donor does, then the recipient does in heat were not mated, but subjected to

conceive the transferred embryos. 7 days after CIDR out, the does were prepared for embryo flushing or embryo transfer depending on whether they were used as donor or recipient does using the method which was previously described.

In Group 2, the 35 donor and 158 recipient does (15 Guishan red-bone goat, 20 Yunnan black mountain goat and 158 Saanen goat) were used the same superovulation protocol with the goats in Group 1, nevertheless, the Group 1 experiment achieved in Kuala Lumpur, Malaysia, and the Group 2 experiment achieved in Kunming, China.

3.5.3 Effect of Repeating Superovulation in Donor Does on Number of Embryo Recovery, Embryo Development Stage and Quality (Kunming) (Experiment 3)

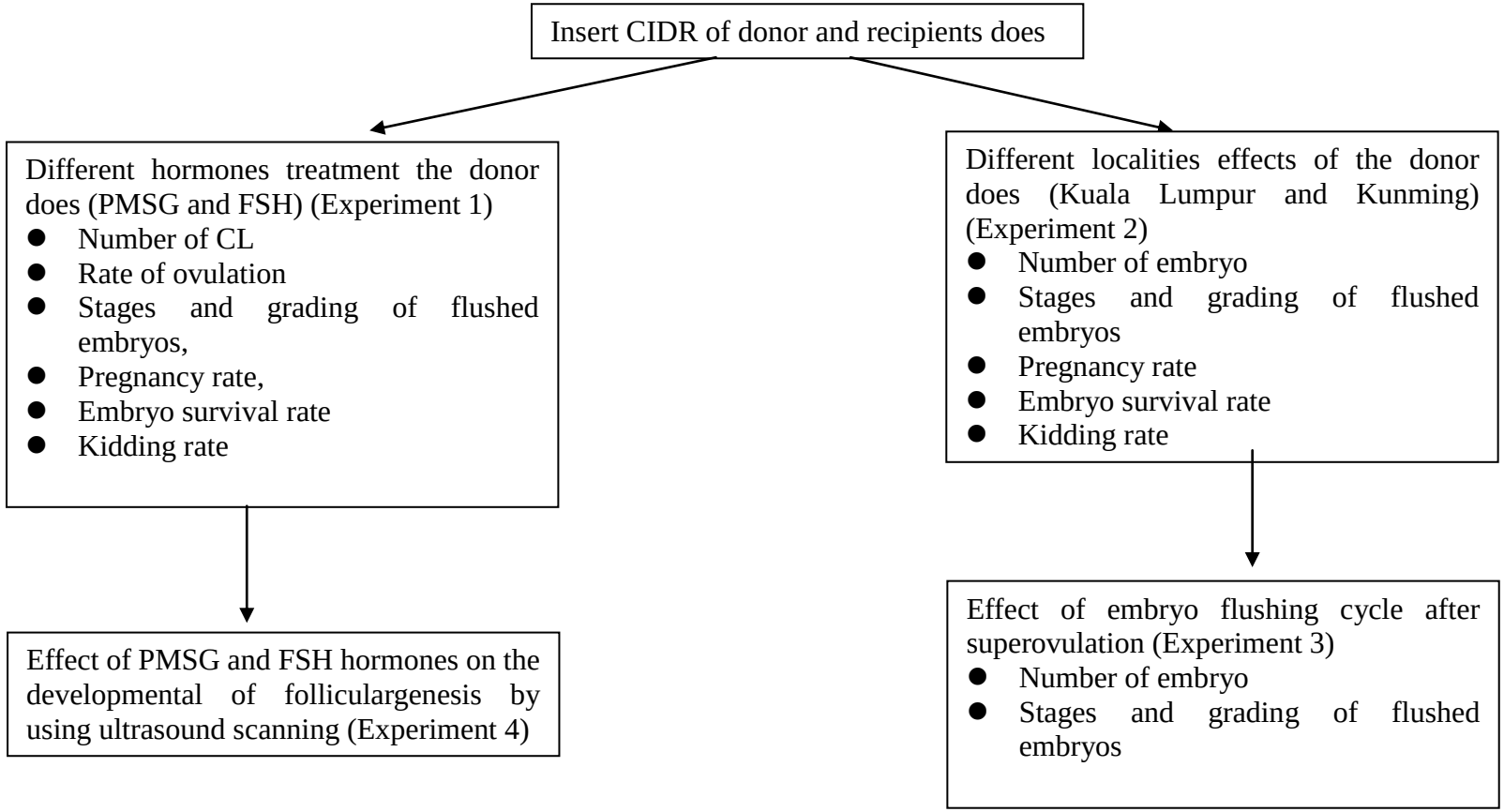
A total of 11 donor does (Guishan red-bone goat) were superovulated using CIDR (17 days) plus FSH-V (160 mg) after which embryos were recovered by flushing technique on day 7 after CIDR withdrawal. The embryos were searched and classified according to stages of embryo development and grades of embryos. The effects of repeating superovulation in does on embryo recovery, embryo development stage and quality were determined**.**

3.6 STATITICAL ANALYSIS

Number of CL, number of embryo obtained, stages and grading of flushed embryos were recorded for analysis using the one-way ANOVA to compare the significant levels between the 3 regimes. The effects of different factors on ET were compared and significant differences between the means were further analysed using Duncan's Multiple Range Test (DMRT) to show the specific differences among the factors on the parameter measured; and $P<0.05$ was considered significant for all statistical tests. The data presented in various experiments were as mean±standard error of means

(mean±SEM). The analysis was carried out with the SPSS (Statistical Packages for Social Science) version 17, SPSS Inc, USA.

3.7 FLOW CHART OF EXPERIMENTAL DESIGN



Chapter 4

4.0 RESULTS

Chapter 4

4.0 RESULTS

4.1 COMPARISON OF PMSG AND FSH-V EFFECTS IN SUPEROVULATION RESPONSES ON EMBRYO RECOVERY RATE, PREGNANCY RATE AND KIDDING RATE IN GOAT AFTER EMBRYOS TRANSFER (KUALA LUMPUR) (EXPERIMENT 1)

A total of 64 donor does were assigned in 2 groups: a) Group 1: CIDR (14 days) plus Estrumate (125 µg), PMSG (1200 IU) and hCG (500 IU), consisting of 47 donor does (South African Boer x Katjang), and b) Group 2: CIDR (17 days) plus FSH-V (160 mg), consisting of 17 donor does (South African Boer x Katjang). Total of 19 recipient does (South African Boer x Katjang) were treated with CIDR (14 or 17 days for Group 1 or 2, respectively) plus PMSG (300 IU), consisting of 9 recipient does for Group 1 and 10 recipient does for Group 2.

For Group 1, the success rates for donor and recipient does in oestrus after synchronisation and superovulation treatment were 85.11% (40/47) and 100% (9/9); for Group 2, the percentage success rates for donor and recipient does in oestrus after treatment were 94.12% (16/17) and 100% (10/10), respectively (Tables 4.1 and 4.4).

For Group 1, donor does were found to have had an ovulation (95%, 38/40) with at least one CL observed in the ovary, whereas only 66.67% (6/9) of the recipients in oestrus were found to have had an ovulation; for Group 2, donor does were found to have had an ovulation of 100% (16/16) with at least one CL observed in the ovary, and 90% (9/10) of the recipients in oestrus were found to have had an ovulation.

In Experiment 1, a total of 311 CL were observed from 38 donor (PMSG) does and a total of 184 CL were observed from 16 donor does (FSH-V). The highest number of CL was observed in donor does treated by FSH-V with 11.50±1.38 per doe and was no

significantly different (P>0.05) from donor does which were treated with PMSG (7.76±1.18). After flushing, a total 106 unfertilised oocytes plus embryos were obtained from Group 1 (38 donor does) and a total 81 unfertilised oocytes plus embryos were obtained from Group 2 (16 donor does), and the percent unfertilised oocytes plus embryos recovered were 34.08% and 43.55%, respectively. Even though insignificance higher number of unfertilised oocytes plus embryos was obtained in donor does treated with FSH-V with 5.06±0.90 per doe which treated with PMSG 2.79±0.66 per doe. A total number of 25 and 70 embryos were obtained from the Groups 1 and 2 of donor does, respectively. The Group 2 donor does were found to have more fertilised oocytes (86.42%) compared with Group 1 (23.58%), and the number of fertilised oocytes per ovulated doe (4.38 ±0.85) compared with Group 1 (0.63±0.23) (P≤0.05) (Table 4.1).

After embryo flushing, the embryos were categorised into different stages (2-, 4-, 8-, 16 cell stages, early morula, compact morula, early blastocyst, mid-blastocyst, fully expanded blastocyst, hatching blastocyst and hatched blastocyst) and gradings (A, B and C) (Figure 4.1 to 4.4). For Group 1, 60% embryos were in early stages (2-, 4-, 8-, 16 cell stages), 20% in morula stages and 20% in blastocyst stages; and 96% of embryos in the good grade (A: 56%, B: 40%), only 4% embryos in the Grade C. For Group 2, the values were 2.86, 48.57 and 48.57% for stage of embryos, and were 55.71, 38.57 and 5.71% for the Grade A, B and C, respectively (Table 4.2 and 4.3).

For Group 1, 14 embryos were transferred to 6 recipient does, whereby, only 2 recipient does were pregnant and successfully delivered 3 kids (Figures 4.5 and 4.6). The pregnancy rate and kidding rate were 33.33 and 50%, respectively; for Group 2, a total of 29 embryos were transferred to 9 recipient does, whereby only 1 recipient doe was pregnant and no kid was delivered. The pregnancy rate and kidding rate were 11.11 and 0%, respectively (Tables 4.3).

In addition, a total of 30 superovulated donor does from Groups 1 (15 does) and 2

(15 does) were further used for the evaluation of follicular development using ultrasound scanning technique.

For Group 1, days 2 and 4 after oestrus and surgery day, 1.40±0.41, 3.13±0.45 and 6.47±1.68 CL per doe were observed, respectively (Figure 4.7 and 4.8); and was significantly different ($P \leq 0.05$) from Group 2 (3.40±0.34, 4.00±0.37 and 11.93±1.50) (Figure 4.9 and 4.10); higher number of unfertilised oocytes plus embryos was obtained in donor does treated with FSH-V with 5.40±0.89 per doe ($P > 0.05$) than donor does which treated with PMSG (3.73±1.16) per doe; however, higher number of embryos were obtained in donor does treated with FSH-V with 4.67±0.85 per doe ($P \leq 0.05$) than donor does which treated with PMSG (0.73±0.40) per doe (Table 4.7).

Table 4.1: Comparison of PMSG and FSH-V effects on oestrus synchronisation and superovulation responses (Experiment 1)

Goats	Group 1 Donor does (PMSG, Kuala Lumpur)	Group 2 Donor does (FSH-V, Kuala Lumpur)
No. of does treated	47	17
Percent does in oestrus (%)	85.11% (40/47)	94.12% (16/17)
Percent does ovulated (%)	95% (38/40)	100% (16/16)
No. of CL	311	184
Percent unfertilised oocytes plus embryos recovered (%)	34.08% (106/311)	43.55% (81/186)
No. of CL / doe in oestrus	7.76±1.18[a]	11.50±1.38[a]
No. of CL / ovulated doe	8.18±1.21[a]	11.50±1.38[a]
Percent oocytes fertilised (%)	23.58% (25/106)	86.42% (70/81)
Unfertilised oocytes plus embryos recovered / ovulated doe	2.79±0.66[a]	5.06±0.90[a]
No. of embryos/ovulated doe	0.63±0.23[a]	4.38±0.85[b]

[a.b] Means with different superscripts in a row were significantly different ($P \leq 0.05$).

Table 4.2: Total oocytes and embryos recovered after flushing at different embryo stages for different embryo qualities (PMSG, in Kuala Lumpur)

Stage	Unfertilised oocytes	2 cell	4 cell	8 cell	16 cell	Early morula	Compact morula	Early blastocyst	Mid-blastocyst	Fully expanded blastocyst	Hatching blastocyst	Hatched blastocyst	Percentage
Grading	81	A: 2	A: 2	A: 4	A: 2	A,1	A:1	A:1	A:1	A:0	A:0	A:0	56%
		B:1	B:0	B:2	B:2	B:2	B:1	B:1	B:0	B:1	B:0	B:0	40%
		C:0	C:0	C:0	C:0	C:0	C:0	C:1	C:0	C:0	C:0	C:0	4%
Total	81	3	2	6	4	3	2	3	1	1	0	0	-
Percent of total embryos	---	60%				20%		20%					100%

Table 4.3: Total oocytes and embryos recovered after flushing at different embryo stages for different embryo qualities (FSH-V, in Kuala Lumpur)

Stage	Unfertilised oocytes	2 cell	4 cell	8 cell	16 cell	Early morula	Compact morula	Early blastocyst	Mid-blastocyst	Fully expanded blastocyst	Hatching blastocyst	Hatched blastocyst	Percentage
Grading	11	A:0	A:0	A:1	A:0	A,2	A:17	A:3	A:3	A:9	A:2	A:2	55.71%
		B:0	B:0	B:1	B:0	B:2	B:11	B:2	B:5	B:4	B:1	B:1	38.57%
		C:0	C:0	C:0	C:0	C:0	C:2	C:0	C:1	C:1	C:0	C:0	5.71%
Total	11	0	0	2	0	4	30	5	9	14	3	3	-
Percent of total embryos	---	2.86%				48.57%		48.57%					100%

Table 4.4: Comparison of PMSG and FSH-V effects on oestrus synchronisation responses, pregnancy rate and kidding rate in goat after embryo transfer

	Group 1	Group 2
Goats	Recipient does	Recipient does
No. of does treated	9	10
Percent does in oestrus (%)	100% (9/9)	100% (10/10)
Percent does ovulated (%)	66.67% (6/9)	90%(9/10)
No. of embryos transfer	14 (transferred embryo from PMSG donor Kuala Lumpur)	29 (transferred embryo from FSH-V donor Kuala Lumpur)
Pregnancy rate (%)	33.33% (2/6)	11.11% (1/9)
Kidding rate (%)	50%	0

Table 4.5: The detailed evaluation of follicular development with PMSG treatment

Donor does ID	Ovary	No. of CL			No. of unfertilised oocytes / embryos obtained	No. of embryos obtained
		2 days after CIDR withdrawn	4 days after CIDR withdrawn	Observed in surgery day		
M0022	Left	0	0	0	0	0
	Right	0	0	0		
K0003	Left	0	2	0	0	0
	Right	-	0	0		
G18004	Left	0	0	1	0	0
	Right	1	1	1		
P0031	Left	0	0	0	0	0
	Right	0	0	0		
P0008	Left	0	0	0	0	0
	Right	0	0	0		
B0067	Left	1	1	2	2	0
	Right	2	3	12		
B0071	Left	-	-	1	6	5
	Right	2	2	3		
B0040	Left	2	3	7	12	0
	Right	2	2	10		
B0037	Left	2	3	4	2	0
	Right	1	1	3		

(Continued)

(Continued)

Donor does ID	Ovary	No. of CL			No. of unfertilised oocytes / embryos obtained	No. of embryos obtained
		2 days after CIDR withdrawn	4 days after CIDR withdrawn	Observed in surgery day		
0143	Left	2	3	8	11	3
	Right	1	1	6		
B0083	Left	2	2	4	7	0
	Right	2	2	13		
G108008	Left	0	1	3	1	0
	Right	0	0	0		
B0036	Left	0	0	1	3	0
	Right	0	1	4		
B0043	Left	0	1	4	11	3
	Right	1	2	8		
0126	Left	0	1	1	1	0
	Right	0	0	1		

Table 4.6: The detailed evaluation of follicular development with FSH-V treatment

Donor does ID	Ovary	No. of CL			No. of unfertilised oocytes / embryos obtained	No. of embryos obtained
		2 days after CIDR withdrawn	4 days after CIDR withdrawn	Observed in surgery day		
0131	Left	1	1	2	0	0
	Right	1	1	2		
0119	Left	2	3	4	4	4
	Right	3	4	8		
0025	Left	2	3	8	2	1
	Right	-	-	1		
0060	Left	1	1	2	3	3
	Right	1	1	2		
0003	Left	1	1	2	4	4
	Right	3	3	5		
0208	Left	1	2	6	5	5
	Right	1	1	4		
B0182	Left	2	3	6	11	11
	Right	-	-	6		
0150	Left	2	3	7	8	3
	Right	2	2	4		
0003	Left	3	3	13	13	11
	Right	2	2	8		

(Continued)

(Continued)

Donor does ID	Ovary	No. of CL			No. of unfertilised oocytes / embryos obtained	No. of embryos obtained
		2 days after CIDR withdrawn	4 days after CIDR withdrawn	Observed in surgery day		
00060	Left	2	3	5	3	1
	Right	1	1	3		
0208	Left	2	2	13	8	8
	Right	2	2	3		
0025	Left	-	-	6	5	5
	Right	3	4	13		
0201	Left	2	2	6	3	3
	Right	1	1	3		
0018	Left	2	3	8	7	6
	Right	2	2	5		
0056	Left	2	2	10	5	5
	Right	4	4	12		

Table 4.7 Comparison of PMSG and FSH-V effects in follicles ovulation

The type of hormone treated	No. of CL/doe			No. of unfertilised oocytes / embryos obtained	No. of embryos obtained
	2 days after CIDR withdrawn	4 days after CIDR withdrawn	Observed in surgery day		
PMSG	1.40±0.41[a]	3.13± 0.45[a]	6.47±1.68[a]	3.73±1.16[a]	0.73±0.40[a]
FSH-V	3.40±0.34[b]	4.00± 0.37[b]	11.93±1.50[b]	5.40±0.89[a]	4.67±0.85[b]

[a.b] Means with different superscripts in a column were significantly different (P≤ 0.05).

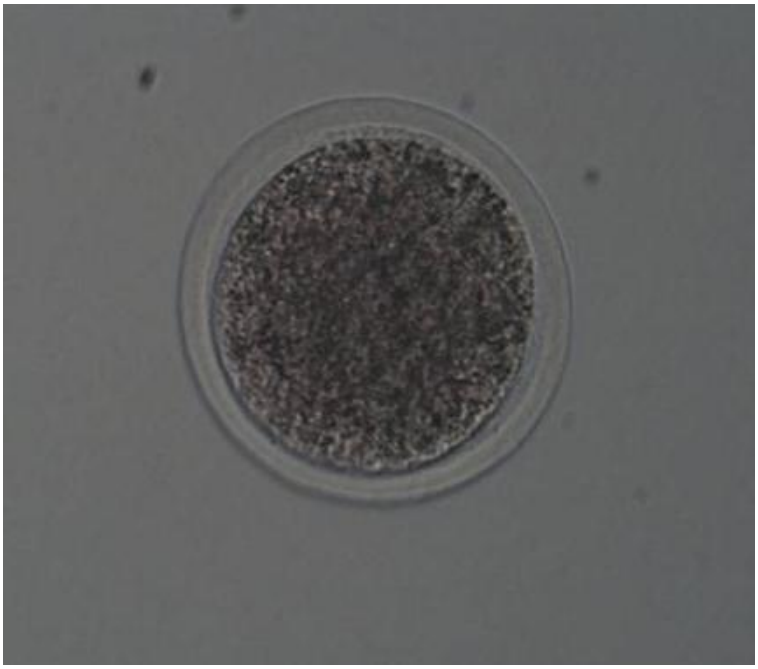


Figure 4.1: Unfertilised oocyte.

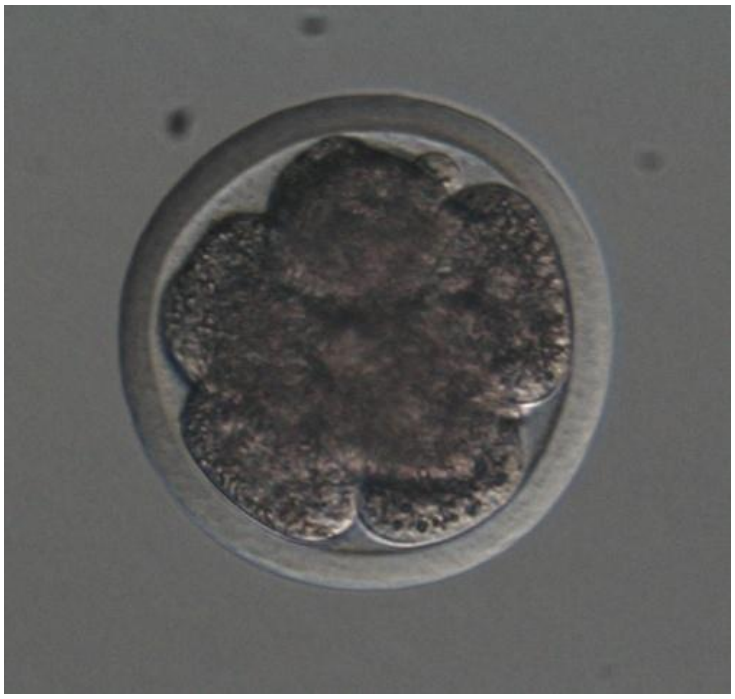


Figure 4.2: 8-cell stage embryo.

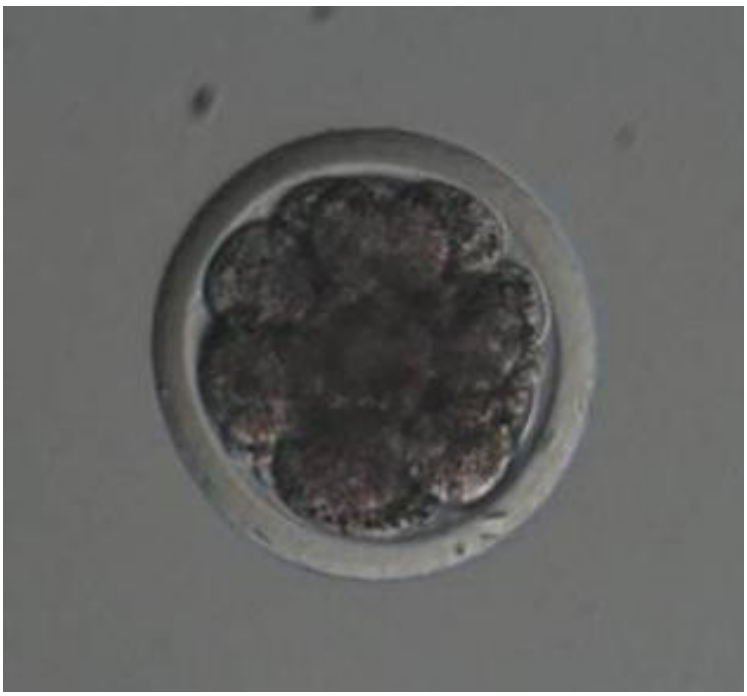


Figure 4.3: 16-cell stage embryo.

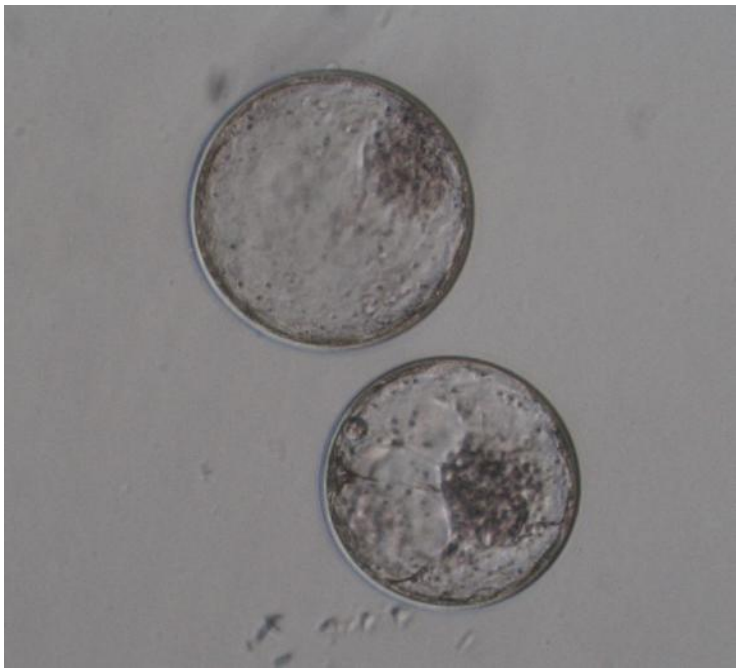


Figure 4.4: Blastocyst stage embryos.

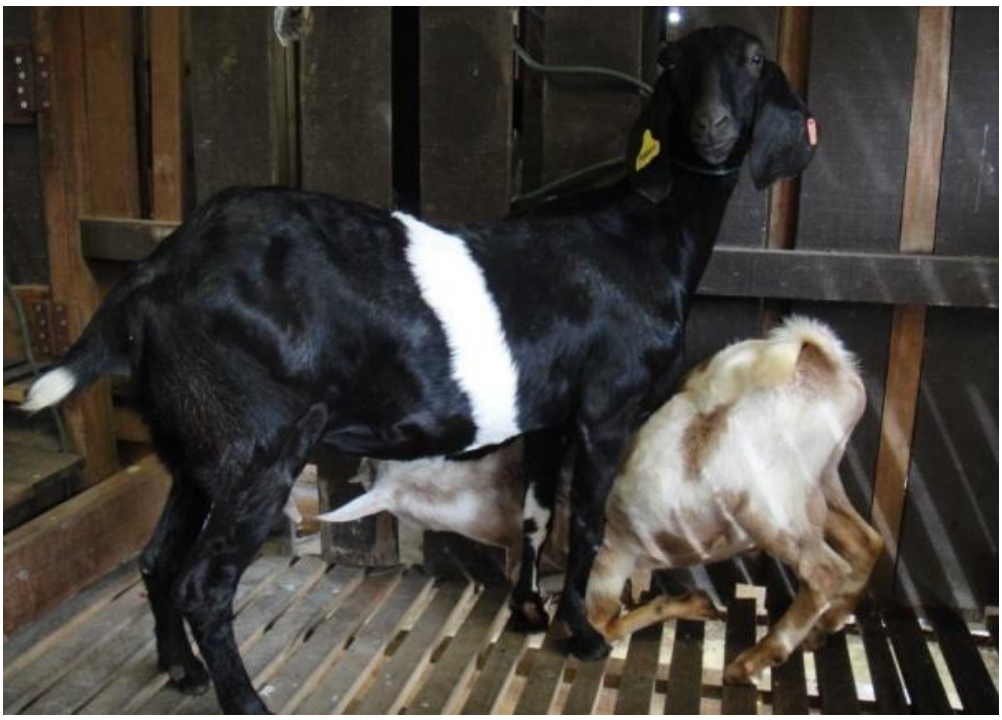


Figure 4.5: The crossbred kid with Katjang recipient doe after embryo transfer using PMSG for superovultion (Kuala Lumpur).

Figure 4.6: The crossbred kid with crossbred recipient doe after embryo transfer using PMSG for superovultion (Kuala Lumpur).

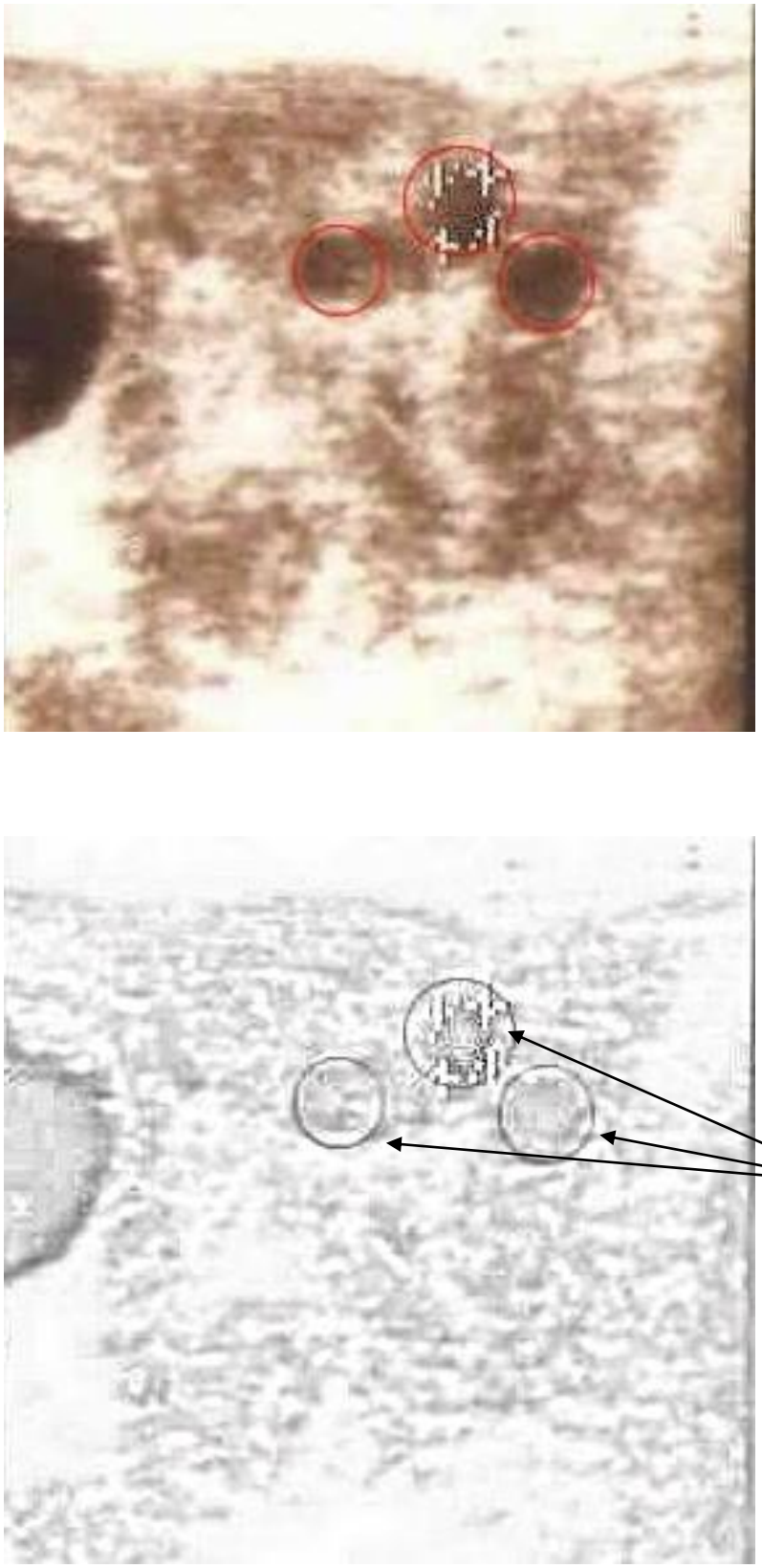


Figure 4.7: The follicles on goat ovary was scanned 2 days after oestrus by using ultrasound scan (treated with PMSG).

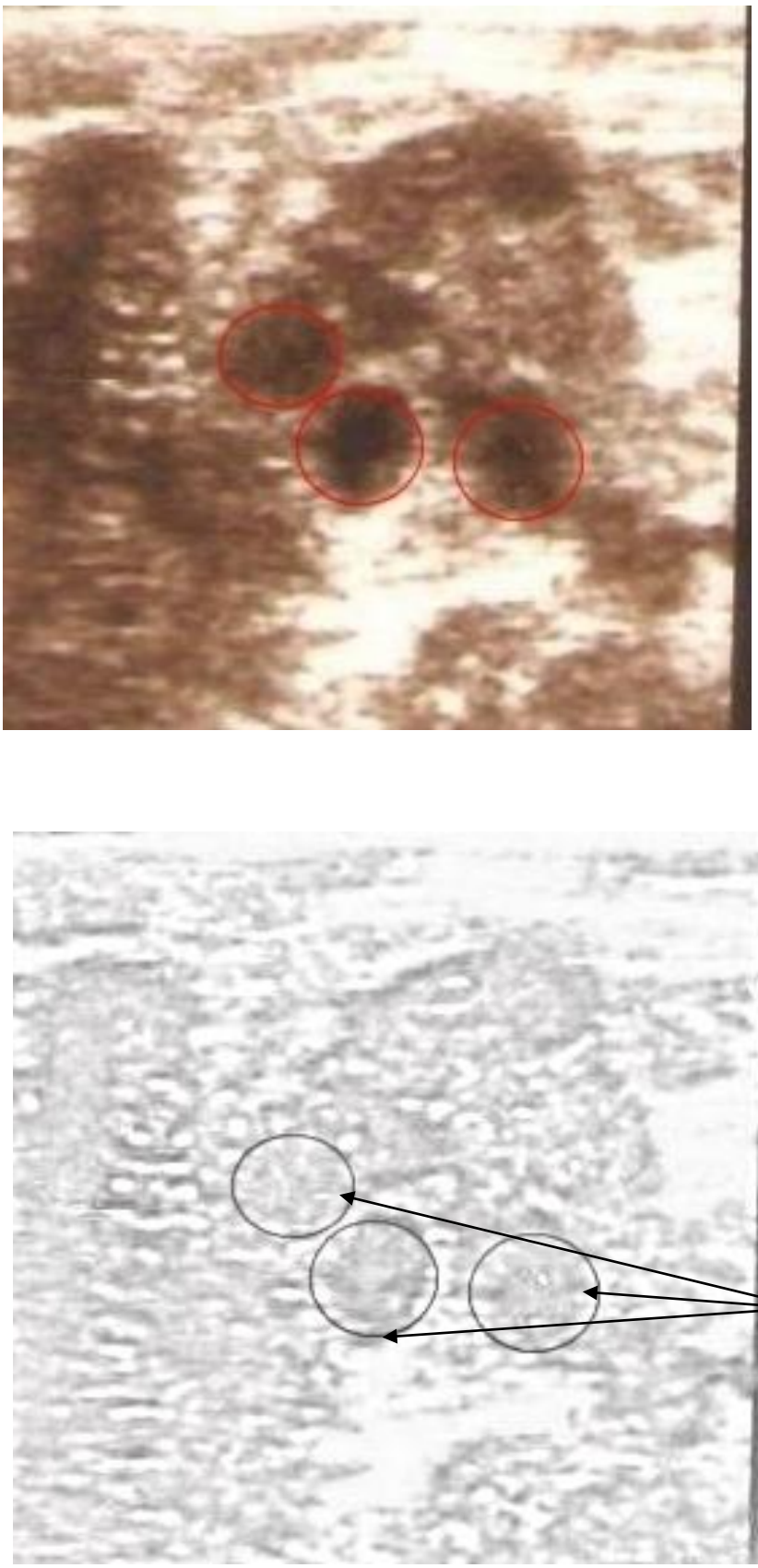


Figure 4.8: The follicles on goat ovary was scanned 4 days after oestrus by using ultrasound scan (treated with PMSG).

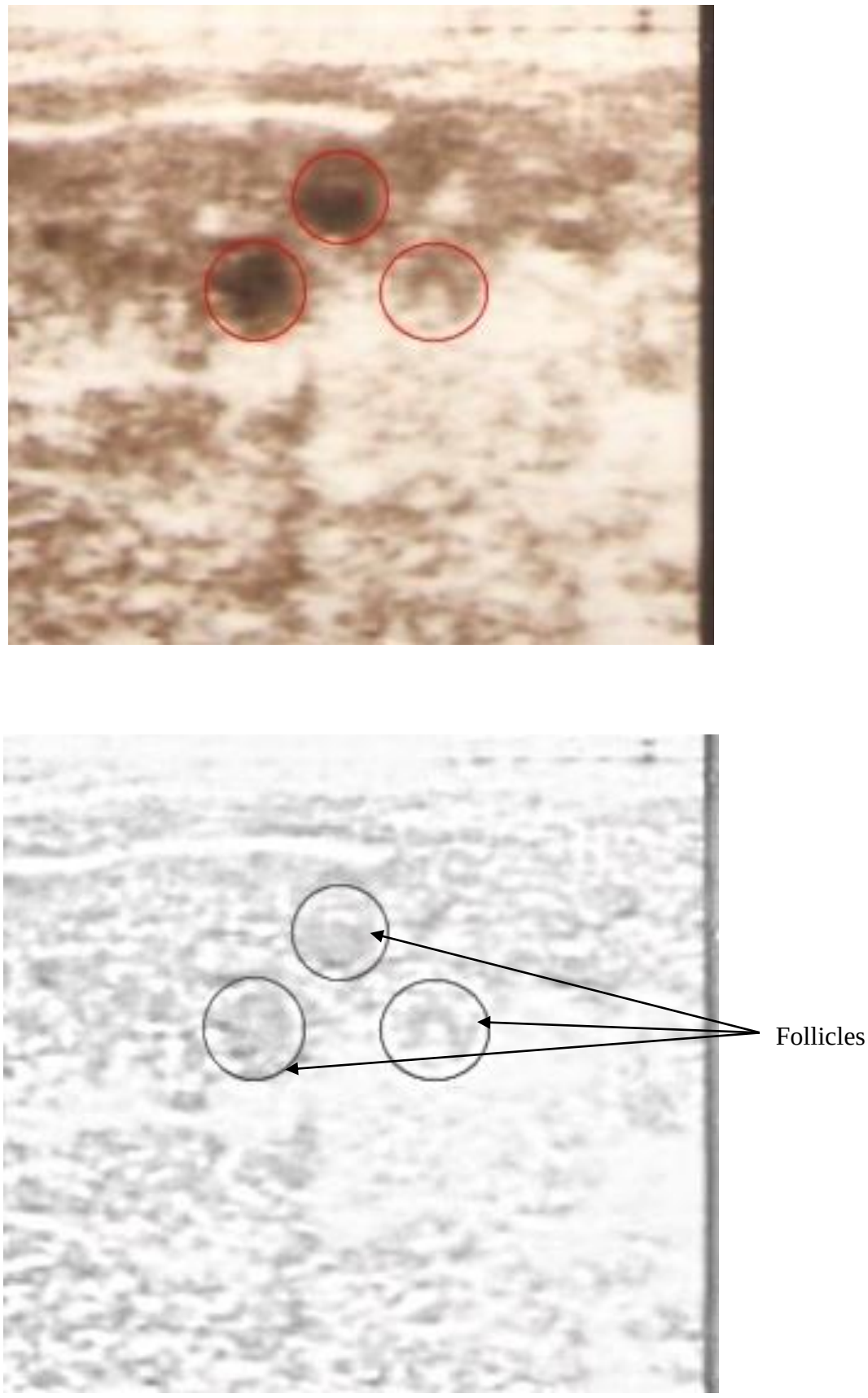


Figure 4.9: The follicles on goat ovary was scanned 2 days after oestrus by using ultrasound scan (treated with FSH-V).

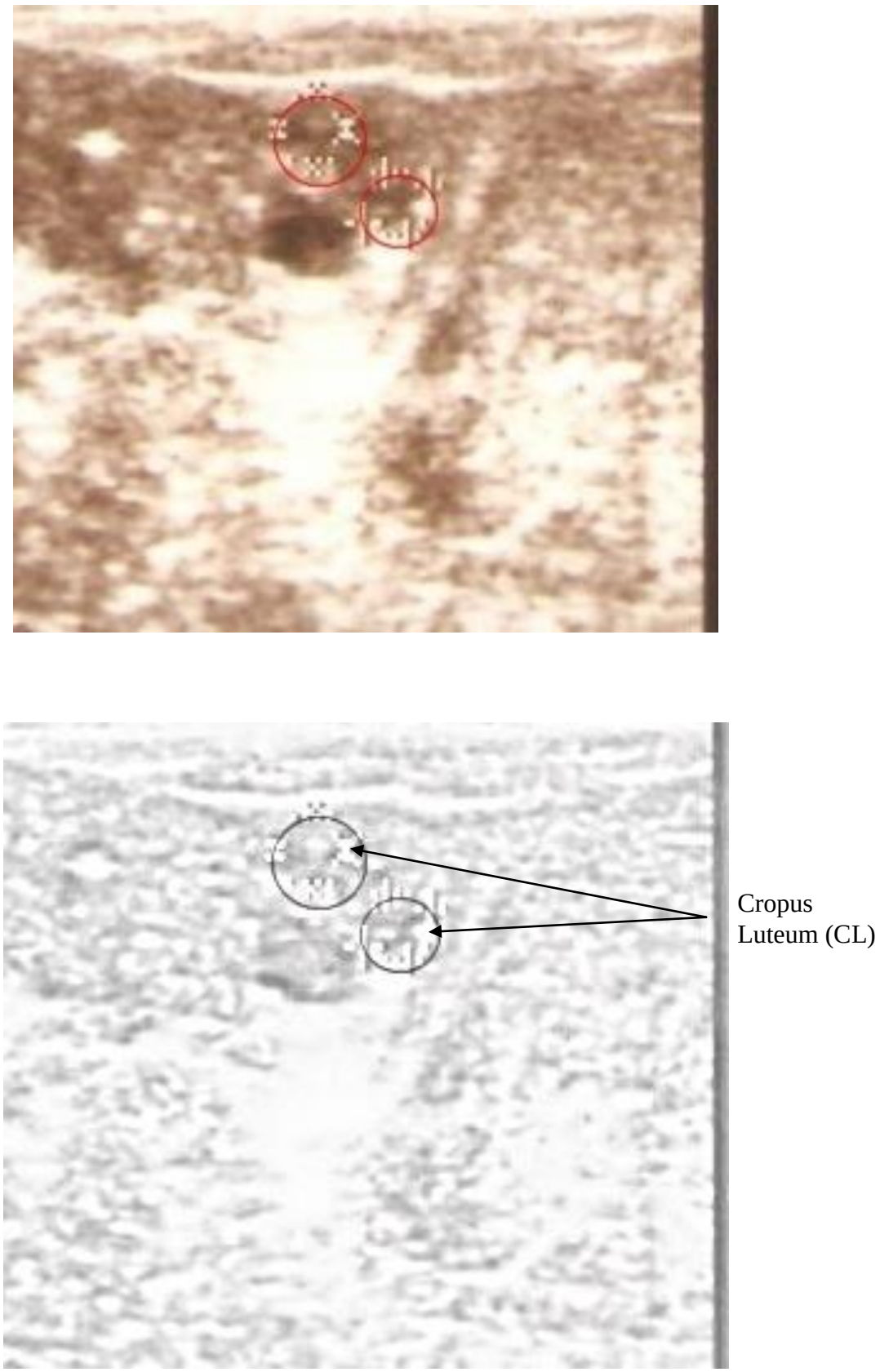


Figure 4.10: The CL on goat ovary was scanned 4 days after oestrus by using ultrasound scan (treated with FSH-V).

4.2 EFFECT OF DIFFERENT LOCALITIES IN EMBRYO TRANSFER PROGRAMME ON SUPEROVULATION RESPONSES, PREGNANCY RATE, AND KIDDING RATE (KUALA LUMPUR AND KUNMING) (EXPERIMENT 2)

A total of 52 donor does were assigned in 2 groups: a) Group 1: CIDR (17 days) plus FSH-V (160 mg), consisting of 17 donor does (South African Boer x Katjang) in Kuala Lumpur and b) Group 2: CIDR (17 days) plus FSH-V (160 mg), consisting of 35 donor does (20 Yunnan black mountain goat and 15 Guishan red-bone goat) in Kunming. The 15 Guishan red-bone donor does were repeatedly used for 3 embryo flushing cycle (15 does in 1^{st} cycle, 12 does in 2^{nd} cycle and 11 does in 3^{rd} cycle). Therefore, the total number of treated donor does was 58 in Kunming. A total of 168 recipient does were treated with CIDR (17 days for both groups in Kuala Lumpur and Kunming, respectively) plus PMSG (300 IU), consisting of 10 recipient does (African Boer x Katjang) for Group 1 and 158 recipient does (Saanen) for Group 2.

For Group 1, the success rates for donor and recipient does in oestrus after treatment were 94.12% (16/17) and 100% (10/10); for Group 2, the success rates for donor and recipient does in oestrus after treatment were 87.93% (51/58) and 93.67% (148/158), respectively (Table 4.8 and 4.10).

For Group 1, donor does were found to have had an ovulation of 100% (16/16) with at least 1 CL observed in the ovary, and 90% (9/10) of the recipients in oestrus were found to have had an ovulation; for Group 2 donor does, were found to have had an ovulation (80.39% 41/51) with at least 1 CL observed in the ovaries; whereas, only 68.92% (102/148) of the recipients in oestrus were found to have had an ovulation.

Comparing localities of embryo transfer using FSH-V for superovulation, the ovarian responses in Kunming gave a significantly higher value ($P \leq 0.05$) (9.47±0.64 unfertilised oocytes plus embryos per doe) than in Kuala Lumpur (5.06±0.90 unfertilised

oocytes plus embryos per doe). A total of 70 and 365 embryos were obtained from Groups 1 and 2 donor does, in which the fertilisation rates were relatively similar between 2 localities (86.42%, 70/81 vs. 85.88%, 365/425); however, the number of fertilised oocytes per ovulated doe for Group 1 (4.38±0.85) was significantly lower ($p \leq 0.05$) compared to Group 2 (8.13±0.69) (Table 4.8).

After embryo flushing, the embryos were categorised into different stages (2-, 4-, 8-, 16 cell stages, early morula, compact morula, early blastocyst, mid-blastocyst, fully expanded blastocyst, hatching blastocyst and hatched blastocyst) and gradings (A, B and C). For Group 1, 2.86% embryos were in early stages (2-, 4-, 8-, 16 cell stages), 48.57% in morula stages and 44.29% in blastocyst stages; for Group 2, the values were 1.10, 45.21 and 53.70%, respectively (Table 4.9) and most of the embryos in the good grade (A: 56 vs. 51%, B: 40 vs.41%, respectively).

For Group 1, 29 embryos were transferred to 9 recipient does, whereby, only 1 recipient does was pregnant and no kid was delivered. The pregnancy rate and kidding rate were 11.11 and 0%, respectively; for Group 2, a total of 131 embryos were transferred to 102 recipient does, 67 recipient does were pregnant and 73 kids were successfully delivered (Figure 4.11 and 4.12). The pregnancy rate and kidding rate were 65.69 and 71.57%, respectively (Table 4.10).

Table 4.8: Comparison of different localities effects in embryo transfer programme on oestrus synchronisation and superovulation responses, pregnancy rate, and kidding rate (Kuala Lumpur and Kunming) (Experiment 2)

Goats	Group 1 Donor does (FSH-V, Kuala Lumpur)	Group 2 Donor does (FSH-V, Kunming)
No. of treated does	17	58
Percent does in oestrus (%)	94.12% (16/17)	87.93% (51/58)
Percent ovulated does (%)	100% (16/16)	80.39% (41/51)
Percent fertilised oocytes (%)	86.42% (70/81)	85.88% (365/425)
Unfertilised oocytes plus embryos recovered / ovulated doe	5.06 ± 0.90^{a}	9.47 ± 0.64^{b}
No. of embryos/ovulated doe	4.38 ± 0.85^{a}	8.13 ± 0.69^{b}

[a.b] Means with different superscripts in a row were significantly different ($P\leq 0.05$).

Table 4.9: Total oocytes and embryos recovered after flushing at different embryo stages for different embryo qualities (FSH-V, in Kunming)

Stage	Unfertilised oocytes	2 cell	4 cell	8 cell	16 cell	Early morula	Compact morula	Early blastocyst	Mid-blastocyst	Fully expanded blastocyst	Hatching blastocyst	Hatched blastocyst	Percentage
Grading	60	A:1	A:2	A:0	A:1	A:6	A:55	A:3	A:49	A:69	A:0	A:0	50.96%
		B:0	B:0	B:0	B:0	B:4	B:79	B:5	B:18	B:41	B:3	B:0	41.10%
		C:0	C:0	C:0	C:0	C:0	C:21	C:1	C:2	C:5	C:0	C:0	7.95%
Total	60	1	2	0	1	10	155	9	69	115	3	0	-
Percent of total embryos	---	1.10%				45.21%		53.70%					100%

Table 4.10: Comparison of different localities effects on oestrus synchronisation responses, pregnancy rate and kidding rate in goat after embryo transfer by recipient

Goats	Group 1 Recipient does	Group 2 Recipient does
No. of does treated	10	158
Percent does in oestrus (%)	100% (10/10)	93.67% (148/158)
Percent does ovulated (%)	90%(9/10)	68.92% (102/148)
No. of embryos transfer	29 (transferred embryo from FSH-V donor Kuala Lumpur)	131(transferred embryo from FSH-V donor Kunming)
Pregnancy rate (%)	11.11% (1/9)	65.69% (67/102)
Kidding rate (%)	0	71.57% (73/102)

Figure 4.11: The Guishan red-bone goat kids with Saanen recipient doe after embryo transfer using FSH-V for superovultion (Kunming).

Figure 4.12: The Guishan red-bone goat kids (1 year old) after embryo transfer using FSH-V for superovultion (Kunming).

4.3 EFFECT OF REPEATING SUPEROVULATION IN DONOR DOES ON NUMBER OF EMBRYO RECOVERY, EMBRYO DEVELOPMENT STAGE AND QUALITY (KUNMING) (EXPERIMENT 3)

A total of 11 donor does (Guishan red-bone goat) were superovulated using CIDR (17 days) plus FSH-V (160 mg) after which embryos were recovered by flushing technique on day 7 after CIDR withdrawal.

For the first time flushing, 5 unfertilised oocytes and 116 embryos were successfully obtained from 11 donor does; as for second time flushing, 9 unfertilised oocytes and 90 embryos were successfully obtained from 9 donor does and 2 donor does were not responding to superovulation; as for the third time flushing, only 1 donor doe was successfully producing 4 embryos and 2 donor does were producing 11unfertilised oocytes (Table 4.11).

After embryo flushing, the embryos were categorised into different stages {early stages (2-, 4-, 8-, 16 cell), morula (early and compact morula) and blastocyst (early, mid-, fully expanded, hatching and hatched blastocyst)} and they were graded accordingly (Grades A, B and C). For the first flushing, 0.9% embryos were in early stages (2-, 4-, 8-, 16 cell stages), 59.1% in morula stage and 40% in blastocyst stage; for second flushing, the values were 3.75, 62.50 and 33.75% (early, morula and blastocyst stage), respectively; and for third flushing, 100% of embryos got were in blastocyst stage. For the grading of embryos, the first time flushing gave 62.73, 34.55 and 2.73% for Grades A, B and C, respectively; for the second time flushing, the values were 36.25, 36.25 and 17.50%, respectively; and for third time flushing, the values were 50.00, 25.00 and 25.00%, respectively.

Table 4.11: The details of number of unfertilised oocytes and embryos obtained by FSH-V for ovulation treatment for each flushing cycle (Kunming)

Goat ID	First cycle		Second cycle		Third cycle	
	No. of unfertilised oocytes	No. of embryos	No. of unfertilised oocytes	No. of embryos	No. of unfertilised oocytes	No. of embryos
04010	2	9	-	-	-	-
R028	-	12	-	12	-	-
06032	-	11	2	4	-	-
03012	3	7	-	14	11	-
06022	-	13	3	5	-	-
03024	-	10	1	6	-	-
06016	-	5	1	9	-	-
No tag	-	13	-	14	-	4
06020	-	17	-	16	-	-
06008	-	9	-	-	-	-
R026	-	10	2	-	-	-
Total no.	5	116	9	80	11	4

Table 4.12: The first time flushing oocytes and embryos recovered after flushing at different embryo stages for different embryo qualities (FSH-V, in Kunming)

Stage	Unfertilised oocytes	2 cell	4 cell	8 cell	16 cell	Early morula	Compact morula	Early blastocyst	Mid-blastocyst	Fully expanded blastocyst	Hatching blastocyst	Hatched blastocyst	Percentage
Grading	5	A:0	A:1	A:0	A:0	A:7	A:22	A:0	A:19	A:20	A:0	A:0	62.73%
		B:0	B:0	B:0	B:0	B:3	B:30	B:0	B:2	B:1	B:2	B:0	34.55%
		C:0	C:0	C:0	C:0	C:0	C:3	C:0	C:0	C:0	C:0	C:0	2.73%
Total	5	0	1	0	0	10	55	0	21	21	2	0	-
Percent of total embryos	---	0.9%				59.1%		40%					100%

Table 4.13: The second time flushing oocytes and embryos recovered after flushing at different embryo stages for different embryo qualities (FSH-V, in Kunming)

Stage	Unfertilised oocytes	2 cell	4 cell	8 cell	16 cell	Early morula	Compact morula	Early blastocyst	Mid-blastocyst	Fully expanded blastocyst	Hatching blastocyst	Hatched blastocyst	Percentage
Grading	9	A:1	A:1	A:0	A:1	A:5	A:10	A:6	A:8	A:5	A:0	A:0	46.25%
		B:0	B:0	B:0	B:0	B:6	B:17	B:5	B:1	B:0	B:0	B:0	36.25%
		C:0	C:0	C:0	C:0	C:2	C:10	C:1	C:0	C:1	C:0	C:0	17.50%
Total	9	1	1	0	1	13	37	12	9	6	0	0	-
Percent of total embryos	---	3.75%				62.50%		33.75%					100%

Table 4.14: The third time flushing oocytes and embryos recovered after flushing at different embryo stages for different embryo qualities (FSH-V, in Kunming)

Stage	Unfertilised oocytes	2 cell	4 cell	8 cell	16 cell	Early morula	Compact morula	Early blastocyst	Mid-blastocyst	Fully expanded blastocyst	Hatching blastocyst	Hatched blastocyst	Percentage
Grading	11	A:0	A:0	A:0	A:0	A:0	A:0	A:0	A:2	A:0	A:0	A:0	50%
		B:0	B:0	B:0	B:0	B:0	B:0	B:0	B:1	B:0	B:0	B:0	25%
		C:0	C:0	C:0	C:0	C:0	C:0	C:0	C:1	C:0	C:0	C:0	25%
Total	11	0	0	0	0	0	0	0	4	0	0	0	-
Percent of total embryos	---	0				0		100%					100%

Chapter 5

5.0 DISCUSSION

Chapter 5

5.0 DISCUSSION

5.1 COMPARISON OF PMSG AND FSH EFFECTS IN SUPEROVULATION RESPONSES ON EMBRYO RECOVERY RATE, PREGNANCY RATE AND KIDDING RATE IN GOAT AFTER EMBRYO TRANSFER (KUALA LUMPUR) (EXPERIMENT 1)

5.1.1 Oestrus Responses

In Kuala Lumpur, the results obtained from this study showed that exhibited signs of oestrus following both 14 (85.11%) and 17 days (94.12%) synchronisation treatment with intravaginal CIDR, whereby the latter apparently was more effective. It has been reported that 100% response to synchronisation in Boer goat for 17 days of CIDR treatment during the breeding season (Lehloenya, *et al* 2008; Lehloenya and Greyling, 2009). For the Boer goat, during the breeding season, 65 (Greyling and Nest, 2000), 97 (Motlomelo *et al.,* 2002) and 98% (Lehloenya *et al.,* 2005) have been reported. These differences may be attributed to the type and dose of progestagen used or the body condition of the does (Lehloenya, 2008), lower oestrus response in goats has also been observed following the oestrus synchronisation of young does (Drion *et al.,* 2001) or when CIDR was utilised without the supplementation of eCG (Oliveira *et al.,* 2001). The reason for the difference in oestrus responses after 14 and 17 days of CIDR implant is not clear currently.

5.1.2 Superovulatory Response

The ovulation rate as indicate by the presence of CL was apparently higher in FSH-V (43.55%) treated group compared with PMSG treated group. After flushing, the higher number of unfertilised oocytes plus embryos were obtained in donor does treated with FSH-V was not significantly different ($P>0.05$) from donor does which was treated with PMSG. However, the number of embryos were obtained in donor does treated with FSH-V was significantly higher ($P\leq0.05$) than donor does treated with PMSG. In studies by other researchers on comparing the superovulatory responses between FSH and PMSG in goat, the evidence favoured the use of FSH than PMSG (Pampoukidou *et al.*, 1992; Pendleton *et al.*, 1992). Eiamvitayakorn *et al*. (1988) obtained 9.6 embryos per doe by FSH treatment and 5.7 embryos per doe by PMSG treatment. In Japan, Tsunoda and Sugie (1989) obtained 9.4 embryos per doe by FSH treatment and 5.7 embryos per doe by PMSG. The Indian researchers have shown that 16.55 CL were observed from per doe by FSH treatment and 11.70 CL per doe by PMSG treatment (Mahmood *et al*., 1991). In Malaysia, Rosnina *et al*. (1992) treated the goat by FSH and PMSG obtained the embryos 6.8 and 3.0, respectively. In Greece, FSH (21 mg) treatment, 12.5 oocytes per doe and PMSG (1200 IU) 3.88 oocytes per doe were obtained (Pampoukidou *et al.*, 1992). Although, most researchers found that FSH was better than PMSG, the PMSG is still widely used for goat superovulation due to the lower cost and easy availability by PMSG (Pargaonkar *et al.*, 1994). Furthermore, PMSG can be more easily administered than FSH, usually as a single injection of up to 1500-2000 IU, at any rate, the superovulatory responses to PMSG can be quite variable and is usually lower than in a FSH-induced superovulation (Amoah and Gelaye, 1990).

Normally, the FSH products are manufactured from the sheep (oFSH), goat

(oFSH) or pig (pFSH). In this experiment, we used FSH-V (Bioniche), which is a highly purified folltropin extract obtained from carefully selected porcine pituitary glands. Compared with 2 different preparations of FSH (FSH-P, Burns-Biotec and FSH, Sanofi), after using, there was no significant difference on goat superovulation; however, there were differences between different manufacturers in producing the hormone preparation (Zhao, 2008).

Some researchers designed the new methods to reduce the intensity of work, without adverse effect on the embryo production. One method was by injecting the FSH at an interval of 1 day with doubling the dosage, and the results showed that no significant difference was observed when compared with the twice injection per day (8.9 and 10.8 per doe, respectively) (Suyadi *et al.,* 2005). Other method was using PMSG (200 IU; injected at the fourth time of conventional FSH injection) replaced the last 3 FSH injections (standard: total 6 times FSH injections), the oocytes recovery rate was the same for the two groups (Pintado *et al.,* 1998). The simple ET method was by injecting the FSH and PMSG at one time, that is by injecting the NIH-FSH-P1 (Folltropin-V) (80 mg) plus eCG (300 IU) with recovery of 15.7 oocytes per doe (Baldassarre *et al*. 2002, 2003, 2007). Similar results were obtained using the conventional method as indicated by Gibbons *et al*. (2007), who injected NIH-FSH-P1 (Folltropin-V) (60 mg) plus eCG (300 IU) with recovery of 5.6-8.0 embryos per doe.

In the present study, the percentages of unfertilised oocytes plus embryos recovered were 34.08 and 43.55% from PMSG and FSH-V treatment does, respectively. These results were lower than that of Mitchell *et al.* (2002), Gonzalez-Bulnes *et al.* (2003) who achieved 68.70 to 86.93%, respectively. The low recovery rate in this study could be

due to the skill of the operator and other uncontrolled factors such as the health and reproductive status of goat, hormone preparation that might be involved during the experiments. Consequently, some of the does were observed to have more than 20 CL, however, after flushing only a few ova, or sometime no ova obtained. Nellenchulte and Niemann (1992) also found out that their recovery rates were low in does which had high ovulation rates (more than 10 CL) and increased in size of the ovaries. They assumed that this could be due to the fimbria did not fully swaddle these enlarged ovaries and consequently the ova were lost in the abdominal cavity. Also, these differences may be attributed to breed differences which have been previously associated with the prolificacy of a breed, while the flushing technique and nutritional status of the donors could also play a role (Gonzalez-Bulnes *et al.,* 2003). Other reason is that it could be attributed to the CIDR insertion timing with the growing or regressing follicle period and follicular wave, as described in the Figures 5.1 and 5.2 (Rubianes *et al.,* 1996; Rubianes and Menchaca, 2003).

For the percentage of oocytes fertilised, FSH-V treatment does showed significantly higher percent of oocytes fertilised than PMSG treated does (86.42% vs. 23.58%, respectively). For the PMSG treated does, the fertilisation rate was lower in this experiment compared with Hamra *et al.* (1989), Tritschler *et al.* (1991) and Senthil *et al.* (2003) where they have achieved 64, 59 and 91%, respectively; however, for the FSH-V treatment does, the fertilisation rate was higher in this experiment than most of other researchers (Gonzalez-Bulnes *et al.,* 2002; Akinlosotu and Wilder, 1993) with the values of 71 and 64%, respectively. But it was still a bit lower compared with the Senthil *et al.* (2003) who achieved 96 and 90%.

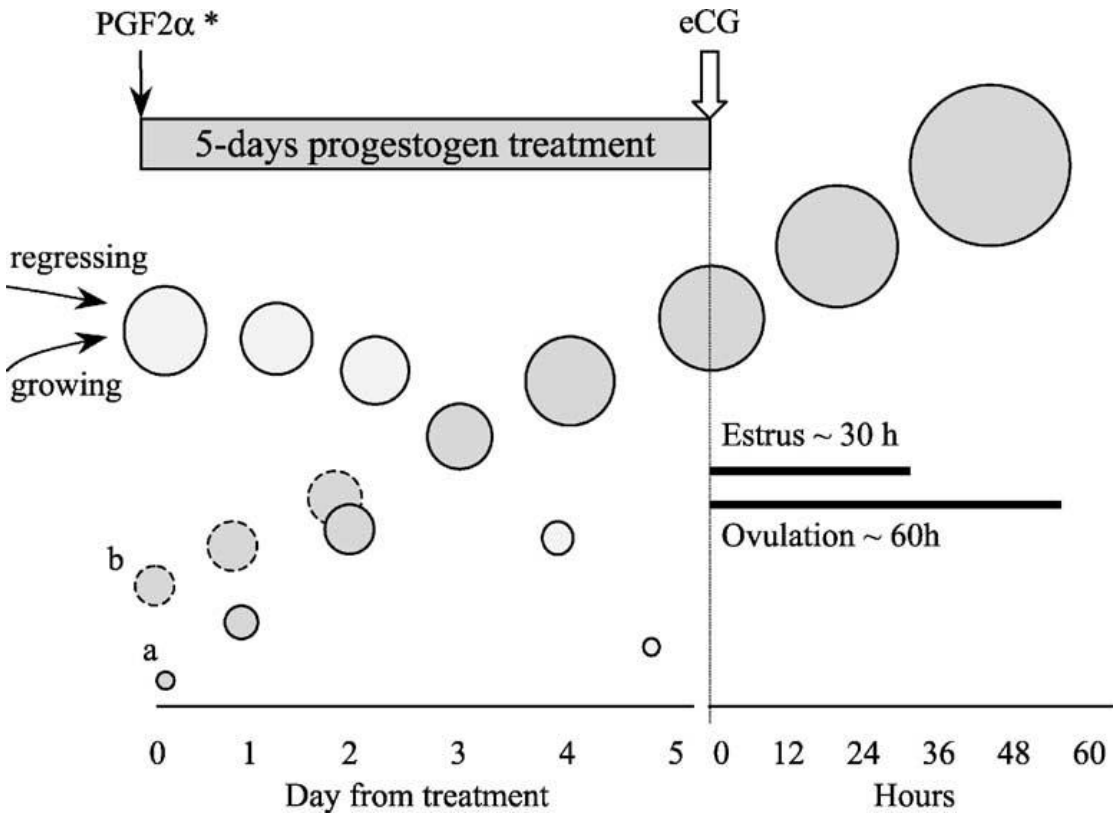


Figure 5.1: Schematic representation of the short priming protocol to induce/synchronise oestrus. The insertion of a progestogen device (asterisk symbol (*) together with a PGF2α dose in cycling goats) promote the regression of the largest follicle (5–10 mm) and the emergence of a new follicular wave (a). Therefore, a young largest follicle (~7 mm) is present at time of device withdrawal that will ovulate around 60 h later. When goats were treated early in the cycle the treatment induced luteal regression and the young largest follicle (3–4 mm) continued to grow and (b) also achieved a pre-ovulatory size (~7 mm) at the time of device withdrawal (Adapted form Rubianes and Menchaca, 2003).

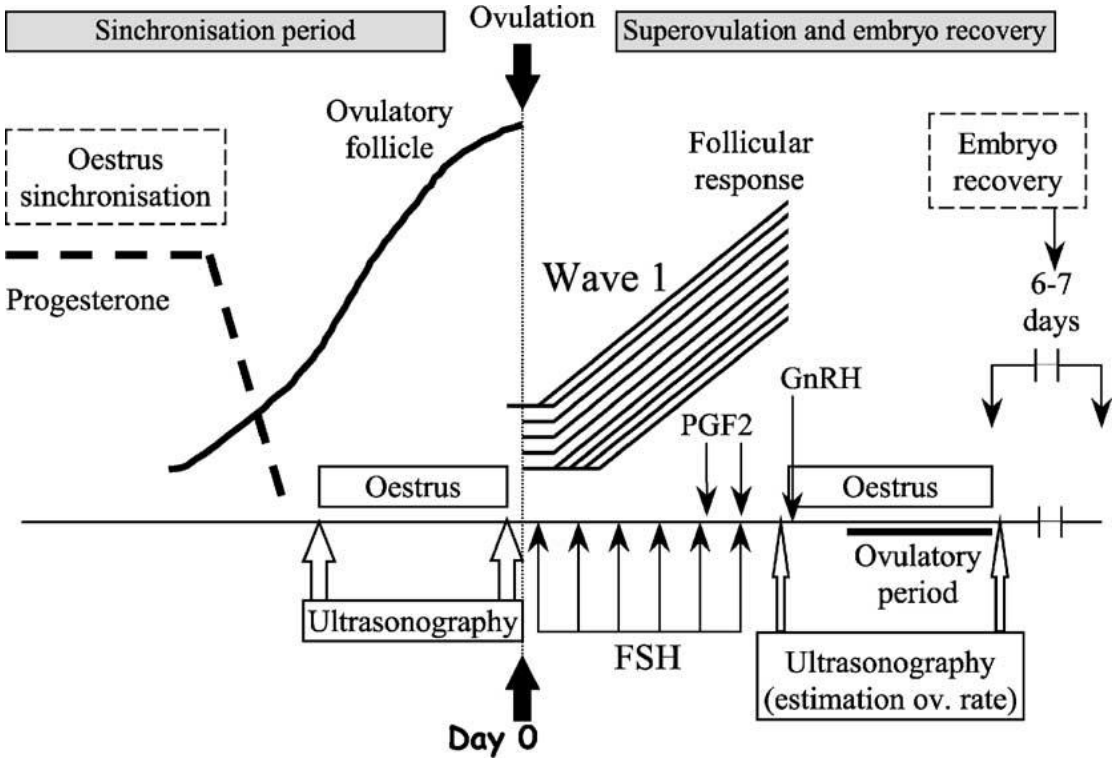


Figure 5.2: Scheme of "day 0 protocol" for MOET used in Uruguayan small ruminant programs. Ovulation (day 0) is determined by transrectal ultrasonography after induce oestrus with progestogen devices or PGF2α. Then, the 6 dose FSH treatment is initiated soon after ovulation (i.e. in absence of a dominant follicle). Alternatively, day 0 can be estimated considering that ovulation occur ~ 36 h after the onset of oestrus. Two half doses of PGF2α are administered together with the 5th and 6th doses of FSH and a dose of GnRH is given at the onset of oestrus (Adapted form Rubianes *et al.*, 1996)

We found that unfertilisation rate of PMSG treatment does was lower than FSH-V treatment does, hence we randomly chose 15 donor does from each group to

further use for the evaluation of follicular development using ultrasound scanning technique. For Group 1, days 2 and 4 after CIDR withdrawn and surgery day, the number of CL observed per doe were 1.40±0.41, 3.13±0.45 and 6.47±1.68, respectively; and were significantly lower ($P \leq 0.05$) from Group 2 (3.4±0.34, 4.00±0.37 and 11.93±1.50, respectively). In this experiment, most donor does exhibited oestrus behaviour 1 day after withdrawal of CIDR. The average oestrus period of the doe was about 40 hours (24-48 hours). When we scanned the goat 2 days after CIDR withdrawn, the most of the does have completed the oestrus, but only 1.40±0.41 and 3.13±0.45 of CL were observed at 2 and 4 days after CIDR withdrawn, respectively. Normally, after oestrus has completed, the buck refused to mount the does. The follicles were observed to ovulate very late (3 or 4 days or even longer after oestrus), which could cause the lower fertilisation rate. It has been reported that the sperm remain viable for only 12 hours in the female reproductive tract, and the life span the ovulated egg is limited to 12-24 hours (Wildeus, 2004). We have injected the does with PMSG plus hCG in order to increase the rate of ovulation, however, the response was negative. The low number of CL were observed could be due to the skill of the operator to identify the structure during the ultrasonic scanning. Furthermore, the ovaries could be observed only one side, which posing difficulties to confirm the absolute number of CL from each ovary. Riesenburg *et al.* (2001) used the ultrasound scanning to observe the oocyte ovulation of the ovary on embryo flushing day. But this method could not judge earlier degeneration of CL and exact number of CL on the ovary. Using the laparoscopic method, it has been shown that there was a high correlation between the number of CL and embryo recovery rate (r=0.9) (Menchaca and Rubianes, 2002). However, in the

present study, using the same skill and operator, the number of CL per doe for donor does treated with FSH-V was significantly higher than the donor does treated with PMSG (Table 4.7).

According to Holtz (1996), the low rate of fertilisation in goat might be due to the slow degradation of eCG, whereby its half-life of 10-15 hours. Treated with pFSH for goat supovulation normally resulted in better responses than PMSG (Nowshari *et al.*, 1992). The main reason could be due to the content of LH, whereby when the LH level reached 40% of its peak, it was not only producing good superovulation, but also good embryo quality. The pFSH half-life only sustained for 5 hours in goats. Therefore, injecting the goat twice per day for 3-4 consecutive days, could obtain 8-16 embryos, however, it depended on individual variation (Holtz, 1996).

The development of embryos was different between the goat and sheep, whereby for the former, most of embryos were at the morula stage 6 days after oestrus, and blastocyst stage at 8 days after oestrus (Table 5.1) (Baril *et al.*, 1993a). The first cleavage was initiated at 12 hours after ovulation, while 2-, 4-, 8-, 16- cell, morula and blastocyst formed 32-48, 48-52, 72-80, 96 hours on days 7-8 after oestrus.

Table 5.1: The different embryo developmental stages after oestrus in goat

The day of embryo collection	The stage of embryo
2	1-2 cell
3	4-8 cell
4	8-20 cell
5	20 cell to early morula
6	Compact morula
7	Mid-blastocyst
	Fully expanded blastocyst
8	Fully expanded blastocyst
	Hatching blastocyst

Compared with this experiment, we flushed all of the donor does 6-7 day after oestrus. However, from the Tables 4.3 and 4.4, the stages of embryo development were significantly different between two hormone treatments. For the PMSG treated does, 60% of the embryos were still at the early stages. It could be due to the delay in the ovulation of the follicles, which were only ovulating about 3 days before flushing. Also it was confirmed by using ultrasound scanning. As mentioned earlier, due to delay of ovulation, the sperm may be dead or lost their fertilisation potential resulting in low fertilisation rate. For the FSH-V treatment does, 91.54% of the embryos were in the morula and blastocyst stage, and this was in agreement with Baril *et al.* (1993a).

For the grades of embryos, most of the embryos were in Grades A and B for the 2 groups (96% and 94.28%, respectively). The better quality of embryos gave a higher pregnancy rate compared with low quality of embryos (Smith and Grimmer, 2002).

5.1.3 Embryo Transfer

Using the laparoscopy method, we successfully transferred 43 embryos to 15 recipients (14 embryos were transferred to 6 recipient does for Group 1, 29 embryos were transferred to 9 recipient does for Group 2, respectively).

McKelvey *et al.* (1985) reported that the goat embryos could be transferred using the laparoscopy method. The advantages of this method are fast (less 5 minutes) and less harmful to the animals. Using the laparoscopy method to transfer embryos, no adhesion for the genital tract, the pregnancy rate is the same with the laparotomy and saves the surgery time (5 minutes vs. 15 minutes) (Vallet *et al.,* 1989). Baril *et al.* (1996) also demonstrated the pregnancy rate was higher or the same when comparing between the laparoscopy and laparotomy methods. It was shown that laparoscopy method was significantly higher in pregnancy rate than exocervical uterine transfer (Flores-Foxworth, 1992).

5.1.4 Pregnancy Rate and Kidding Rate

For the 2 groups, the pregnancy rate and kidding rate were lower than other findings from other researchers. Usually the pregnancy rate after embryo transfer in goat was about 45 to 80%. It could be attributed to the embryo quality, the nutrition of the recipient does and the method of the transfer. If the recipient does lack of the nutrition, it could lead to the low pregnancy rate (Mani *et al.*, 1992). Other reason could be due to the number of CL, a low serum progesterone concentration from a single CL at transfer, thus may have reduced the pregnancy and kidding rates. It has also been observed that the survival rate of embryos was increased by transferring embryos to recipient does with 2, 3 or more CL and a high circulating progesterone concentration (Armstrong and Evans, 1983).

However, recent studies have shown that the number of CL present at transfer did not have any effect on the eventual pregnancy and kidding rates (Baril *et al.,* 2001; El-Gayar and Holtz, 2001).

Comparing the two groups, the pregnancy and kidding rates for Group 1 was significantly higher than Group 2 (33% vs. 11% and 50% vs. 0%, respectively). Grade A embryos were chosen for embryo transfer in this study; therefore, the factor of embryo quality could be excluded for the difference pregnancy and kidding rates. For Group 1, most of the embryos transferred in the early and morula stage compared with Group 2 (most in blastocyst stage). In contrast, the transfer of morula and blastocysts stage always leads to a higher survival rate compared to early stage in ruminants (Baril *et al*., 2001; Guignot *et al*., 2006). For this experiment, the different results may be attributed to nutrition, season and the age of recipient does.

5.2 EFFECT OF DIFFERENT LOCALITIES IN EMBRYO TRANSFER PROGRAMME ON SUPEROVULATION RESPONSES, PREGNANCY RATE, AND KIDDING RATE (KUALA LUMPUR AND KUNMING) (EXPERIMENT 2).

5.2.1 Oestrus Responses

In this experiment, majority of the does showed signs of oestrus following 17 days synchronisation treatment with intravaginal CIDR in Kuala Lumpur (96.29%) and Kunming (92.13%), whereby the former was apparently more effective. In Kunming, a few does did not exhibit oestrus that could be attributed to the age of the does (whereby

some of the does were more than 5 years old) that could lead to the degradation of the body function that would bring about the reproductive disorder in the does.

5.2.2 Superovulatory Responses

In Kuala Lumpur, the does treatment were found more than 96.15% (25/26) of the does had an ovulation with at least 1 CL in the ovaries compared with 71.86% (143/199) in Kunming. There were probably 2 main reasons that could attribute to this phenomenon, namely the age of does and repeated using of donor does for the superovualtion and embryo recovery. After flushing, the number of unfertilised oocytes plus embryos and number of embryos were obtained per donor doe treated in Kunming were significantly ($P \leq 0.05$) higher (9.47±0.64, 8.13±0.69, respectively) than that of in Kuala Lumpur (5.06±0.90, 4.38±0.85, respectively). However, the results of the fertilisation rate were almost the same for the 2 groups. Besides that, the stages of embryos obtained after flushing were also found similar for the 2 groups, whereby almost all embryos were in the morula and blastocyst stages. This may explain that when using FSH for superovulation, the embryos normally ovulate in 30 to 36 hours after oestrus, hence it will facilitate maximum capacity for the oocytes to be fertilised by the sperm.

5.2.3 Pregnancy and Kidding Rates

The pregnancy and kidding rates in Group 2 were significantly higher than that of Group 1. One of the main reasons was observed to be due to poor body condition of the recipient does. For Group 1, the recipient doe body weights ranged from 20 to 30 kg. However, for Group 2, the recipient does body weights ranged from 40 to 50 kg.

5.2.4 Comparison of Different Localities Effect on Embryo Transfer Performance

Geographically, Kuala Lumpur, Malaysia is situated with the latitude and longitude of 3°N 101°E, with tropical climate, no traditional breeding season for animals, rainfall plentiful. Kunming, China, is situated with the latitude and longitude of 25°N 102°E, with sub-tropical monsoon climate, 4 distinct seasons, dry and rainless (Table 5.2). The climate of 2 areas is totally different in which Kunming has the traditional breeding seasons for the does while Kuala Lumpur is typical non-seasonal tropical climate. Therefore this could be the main reason for the effect of different localities on the embryo transfer performance in the goats studied.

The effect of photoperiod on goat reproduction is not obvious compared to sheep. Most of the goats live in the tropical and subtropical zones. In American continent, most of the goat show the obvious traditional breeding season, e.g. the Angora goat is a seasonal breeder, whereby only a few of the goats, especially dairy goats, show oestrus before September. Also, in Australia, the Cashmere goat showed the obvious traditional breeding seasonal (Restall 1992). The goat living the suitable condition, some breed of the goat showed the oestrus whole year, most of goat breed live in tropical and sub-tropical zone showed this feature. The peak progenitive activity of Boer goat showed in autumn, the lowest showed in deep spring and mid-summer (Greyling and Niekerk, 1987).

Table 5.2: Geographical and environmental information of Kuala Lumpur, Malaysia and Kunming, China

	Kuala Lumpur	Kunming
Latitude and longitude	3°N 101°E	25°N 102°E
Average temperature	23.2 to 32.4 °C	10.3 to 20.8 °C
Relative humidity	82%	74%
Sunshine duration	2229.6 hours	2197.6 hours
Season	Traditional season	No traditional
Height above sea level	100 M	1895 M
Climate	tropical climate	Sub-tropical monsoon climate

Comparison on superovulation responses with the different localities, the number of unfertilised oocytes plus embryos as well as number of embryos obtained per donor doe in Kunming were significantly ($P \leq 0.05$) higher (9.47±0.64, 8.13±0.69, respectively) than that of in Kuala Lumpur (5.06±0.90, 4.38±0.85, respectively). The goat oestrous cycle was not only adjusted by the intrinsic factors, e.g. reproductive endocrinology, nervous system but also adjusted by the external factors, e.g. season and nutrition. Some other external factors show their important relationships with goat reproduction, e.g. the changing of the climate, such as photoperiod, temperature, amount of precipitation and humidity. The photoperiod and temperature are the main factors for the climate effects for goat reproduction (Chemineau *et al*., 2004). In south of China, the duration of day was significantly different in the 4 seasons. Consequently, the goats show the obvious seasonal reproduction. The goat seasonal anoestrous degree increases following the goat

breeding area latitude increases (Zhao, 2008). For this experiment, the 2 localities have almost the same longitude (101°E vs. 102°E, respectively), and almost the same sunshine duration (2229.6 vs. 2197.6 hours per year, respectively). Therefore, these 2 factors could not be affecting the goat reproduction between the two localities. However, a difference between Kuala Lumpur and Kunming in latitude (3°N vs. 25°N) and altitude (100 m vs. 1895 m) may influence the ambient temperature and atmospheric oxygen and thus may cause the difference in reproductive physiology of the goats between the two localities. For the 2 localities, the temperature in Kuala Lumpur is almost constant throughout the year (annual average: 23.2 to 32.4°C), while in Kunming it varies according to season (annual average: 10.3 to 20.8°C) (Table 5.2). In Kuala Lumpur, without traditional breeding season, the goats are in oestrus the whole year round. However, in Kunming, the temperature changes significantly throughout the year, especially when comparing between summer and winter temperatures, resulting in traditional breeding season in goats. The natural breeding seasons are spring and autumn in Kunming. For the superovulation responses, there are significant differences between the two localities. It was reported that during the breeding season, the goat shows favorable intrinsic factors (reproductive endocrinology, nervous system) and external factors (photoperiod, temperature, amount of precipitation, humidity and nutrition) that enhance breeding activities in goats (Pendleton *et al.*, 1986; Walker *et al.*, 1989; Sebastian-Lopez *et al.*, 1990; Mitchell *et al.*, 2002; Fatet *et al.*, 2011).

Breeding season of the goat could be adjusted without using hormones. One of the methods is artificial change the photoperiod, e.g. artificially extending the photoperiod in winter could stimulate the goat in oestrus during the non-traditional breeding season

(Ashbrook, 1982). The principle of the photoperiod intervention is through the release of endogenous melatonin. Melatonin, the main secretory product from the pineal gland, transmits a signal of the circannual rhythm, which transduces information about the environmental light received by the retina and regulates seasonal reproduction as well as many other biological processes such as hibernation, migration and pelage changes. In the large number of mammalian species studied, secretion of melatonin occurs mainly or only during the night, while during the day the plasma concentrations are very low or below the detection limit (Arendt, 1985, 1995). Therefore, this neurohormone is able to entrain and synchronise both circadian (Pevet *et al.*, 2006) and circannual endogenous rhythms (Lincoln, 2006).

5.3 EFFECT OF REPEATING SUPEROVULATION IN DONOR DOES ON NUMBER OF EMBRYO RECOVERY, EMBRYO DEVELOPMENT STAGE AND QUALITY (KUNMING) (EXPERIMENT 3)

In the present study, when repeating 3 times of embryos flushing, the oestrus responses, superovulation responses and embryos obtained were showed to be significantly different from the first 2 time flushings (116 embryos to 4 embryos, respectively). Therefore, the number of embryos dramatic declines when superovulation and recovery of embryos were repeated from same donor doe for 3 times and more, which can be a feasible way to optimise *in vivo* embryo production in females that have high genetic value (Forcada *et al.*, 2010). However, repeated superovulation in small ruminants in the past has led to undesirable side effects. Repeated superovulation with pFSH in goats has been reported to reduce the number of ovulations and embryos recovered, the quality of embryo, as well

the number of transferable embryos (Nuti *et al.,* 1987; Baril *et al.,* 1989; Beckers *et al.,* 1990).The similar situation was observed in other species (Cattle, monkey) (Chupin and Saumande, 1979; Al-kamali *et al.,* 1985; Bavister *et al.*, 1986). The anti-eCG antibodies produced in goats have been indicated to have a negative effect on reproduction, especially when fixed-time AI is performed (Roy *et al.*1999). It has been shown that when using high concentration of anti-eCG antibodies, it would result in a decrease in fertility. The reduction in fertility is believed to arise from the alteration in the time of the occurrence of the expected oestrus. It has been observed that the proportion of goats exhibiting a delayed onset of induced oestrus behaviour, increased with the number of eCG treatments (Baril *et al.,* 1993a). Baril *et al.* (1996) treated the goat twice by eCG, the antibody level increased in the blood, the efficiency of this gonadotrophin decreased for the oestrus and superovulation responses. They suggested that the superovulation treatment with eCG should be carried out only once in each doe. In France, many researchers demonstrated that most of goat showed the oestrus 24 to 72 hours after CIDR removal. It has also been shown that no significant difference was observed between the breeds and age of the does. However, for the second repeated superovulation treatment on the doe, the earlier oestrus goat had higher offspring productivity than later oestrus goat after CIDR removal (Leboeuf *et al.,* 1998). A research results showed that the antibody of eCG increased from 10 days after first time eCG injection, and subsequently the concentration of antibody reached the peak at 10 to 17 days after eCG injection and gradually decreased in next 2 months. For the second repeated superovulation treatment, the pattern of antibody change was similar, but the antibody was increased from 7 days after first time eCG injection, and gradual decline took longer period, and therefore

higher concentration antibody in the blood causing the low reproductive performance in the goat (Zhao, 2008). There was no significant difference with FSH repeated treatment for goat. This could be due to the sources of FSH production from sheep and goat have low antibody for the donor does and thus resulting in good superovulation responses (Baril *et al.*, 1992). It has also been suggested that sheep and goat FSH must be utilised if embryos are to be collected from a donor several times (Chemineau *et al.*, 1999). In contrast, for the pFSH repeat treatment, some researchers found that anti-gonadotrophins have frequently been detected in poor and non-responder goats (Beckers *et al.*, 1990; Remy *et al.*, 1991). In addition, the surgery distress imposes numerous disadvantages to the donor does, e.g. surgical trauma during exteriorisation of the reproductive tract through laparotomy and the formation of post-operative adhesions. This limits the number of times surgical flushings could be performed on the same animal (Mckelvey *et al.*, 1985; Ishwar and Memon, 1996; Pereira *et al.*, 1998; Suyadi *et al.*, 2000). For this experiment, the first time and second times of embryo flushing, the oestrus and superovulation responses were not significantly different; however, the third time embryo flushing was significantly different from the previous. Besides the reasons mentioned above, other factor is the third time embryo flushing was done in winter (December), which is not the traditional breeding season for goat in Kunming. Therefore, described in the previous section, this probably is the major reason for the significant decline in embryo production for the third flushing.

5.4 FUTURE DIRECTIONS

The results from this present study provide additional knowledge to existing information regarding the goat oestrus synchronisation, superovulation responses and embryo transfer, there are still room for improvements that can be carried out in the future. Firstly, the goat age and body condition were the very important key for this experiment. The goat age was around 2 to 4 years better and the goat should in the healthy condition supply the enough food for daily. For the oestrus synchronisation treatment with the CIDR, the 17 days CIDR treated better than 14 days. For the superovulation with different hormonal, the FSH-V obtained more embryos than PMSG, therefore, for the superovulation for the embryo transfer, suggestion use FSH-V instead of using PMSG. Also, could try the FSH mix with PMSG treatment method in the future. For the effect of locality, the goat reproductive efficiency in breeding season areas was better than no breeding season areas.

In addition, the ultrasonic scanning could be very useful for detection of the presence of follicles and CL in ovary before insertion CIDR or surgery, which method could observed the development of follicle and CL, estimate the stage follicular wave, then, insertion the CIDR at the optimum time, thereby, obtained the good result form superovulation responses.

5.5 CONSTRAINTS AND SOLUTIONS TO PROBLEMS

In this study there were a few constraints encountered. The lack of experimental does available in Kuala Lumpur leads to the small sample size analysed in this study. Furthermore the poor animal management and feed problems in the farm contributed to the negative effect on the body condition of both donor and recipient does, thus indirectly affect the number of embryos obtained and the success rate of live birth post-ET. In Kunming, some of the does used as donors and recipient were aged around 5 years old due to insufficient goat source for selection. The aging effect of the does might contribute to the lack in efficiency of embryo production and birth of offspring after ET.

The above constraints can be overcome in future by improving the farm management and a proper study to improve the goat feed nutrient can be carried out prior MOET research. If possible, prior experiment, selection of does must be done meticulously to avoid selecting poor reproductive performance does or aged does.

Chapter 6

6.0 CONCLUSIONS

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This research successfully produced goat offspring from *in vivo*-derived embryos through embryo transfer techniques with the main objective of the experiments were achieved.

a) In the oestrus synchronisation, both donor and recipient does synchronised with 17 days of CIDR administration showed a higher ovulation rate compared with 14 days of CIDR treatment.

b) Using laparoscopic embryo transfer technique, offspring were successfully delivered in Kuala Lumpur and Kunming.

c) The suitable age for the recipient does was ranged from 2 to 5 years old and the body weight of more than 30 kg.

d) For the different hormones for superovulation protocol, the ovulation, unfertilised oocytes plus embryos recovery and fertilisation rates were higher in the FSH-V than those of PMSG treated does. The number of embryos obtained in donor does treated with FSH-V per doe was significantly higher than donor does treated with PMSG per doe.

e) The unfertilised oocytes plus embryo recovery and number of embryos obtained per doe were significantly higher in Kunming than those of Kuala Lumpur.

f) For FSH-V treatment, the pregnancy and kidding rates after embryo transfer were higher in Kunming than those of Kuala Lumpur.

g) For FSH-V treatment, for the repeating 3 times of embryos flushing in Kunming, a total number of embryos obtained were shown to be significantly higher from the first 2 time flushings versus the third time flushing.

In conclusion, 17 days CIDR implantation for oestrus synchronisation, FSH-V treatment and repeating 2 times of embryo recovery from donor does for superovulation generally give better performance in ovarian responses and embryo transfer performance, with experiments in Kunming show better results than in Kuala Lumpur. Both localities produce live offspring after embryo transfer.

REFERENCES

REFERENCES

Abdullah, R.B., S.L. Liow, A.N.M.A. Rahman, W.K. Chan, W.E. Wan Khadijah and S.C. Ng. 2008. Prolonging the interval from ovarian hyperstimulation to laparoscopic ovum pick-up iproves oocyte yield, quality and developmental competence in goats. Theriogenology. 70:765-771.

Adamiak, S.J., K. Mackie, R.G. Watt, R. Webb and K.D. Sinclair. 2005. Impact of nutrition on oocyte quality: cumulative effects of body composition and diet leading to hyperinsulinemia in cattle. Biology of Reproduction. 73:918-926.

Adams, G.P., R.L. Matteri and O.J. Ginther. 1992. Effect of progesterone on ovarian follicles, emergence of follicular waves and circulating follicle-stimulating hormone in heifers. The Journal of Reproduction and Fertility. 95:627-640.

Akinlosotu, B.A and C.D. Wilder. 1993. Fertility and blood progesterone levels following LHRH-induced superovulation in FSH-treated anoestrous goats. Theriogenology. 40:895-904.

Al-Kamali, A.A., M.P. Boland, T.F. Crosby and I. Gordon. 1985. Reduced superovulatory response in the ewe following repeated gonadotrophin treatment. Veterinary Record. 116:180-181.

Ammoun, I., T. Encinas, A. Veiga-Lopez, J.M. Ros, I. Contreras, P. Gonzalez-Anover, M.J. Cocero, A.S. McNeilly and Gonzalez-Bulnes. 2006. Effects of breed on kinetics of ovine FSH and ovarian response in superovulated sheep. Theriogenology. 66:896-905.

Amoah, E.A and S. Gelaye. 1990. Superovulation, synchronization and breeding of does. Small Ruminant Research. 3:63-72.

Arendt, J. 1985. Mammalian pineal rhythms. Pineal and Retinal Peptides and Their Receptors. 3:161-213.

Arendt, J. 1995. Melatonin and the Mammalian Pineal Gland. London: Chapman and Hill.

Armstrong, D.T. 2001. Effects of maternal age on oocyte developmental competence. Theriogenology. 55:1303-1322.

Armstrong, D.T. and G. Evans. 1983. Factors affecting success of embryo transfer in sheep and goats. Theriogenology. 19:31-42.

Ashbrook, P.F. 1982. Year-round breeding for uniform milk production. Proceeding of the 3rd International Conference on Goat Production, Scottsdale, Arizona. pp.153-154.

Averill. R.L.W. and L.E.A. Rowson. 1958. Attempts at storage of sheep ova at low temperatures. The Journal of Agricultural Science. 52:392-395.

Baker, R.D. and P.J. Dziuk. 1971. Aerial transport of fertilised pig ova. Canadian Journal of Animal Science. 50:2115–2216.

Baker, R.D., S. Webel, A. Ellicott and P.J. Dziuk. 1971. Aerial transport of sheep ova *in vitro*. Canadian Journal of Animal Science. 51:542–543.

Baldassarre, H. and C.N. Karatzas. 2004. Advanced assisted reproduction technologies (ART) in goats. Animal Reproduction Science. 82-83:255-266.

Baldasssare, H., B. Wang, N. Kafidi, C. Keefer, A. Lazaris and C.N. Karatzas. 2002. Advances in the production and propagation of transgenic goats using laparoscopic ovum pick-up and *in vitro* embryo production technologies. Theriogenology. 57:275-284.

Baldassare, H., C. Keefer, B. Wang, A. Lazaris and C.N. Karatzas. 2003. Nuclear transfer in goats using in vitro matured oocytes recovered by laparoscopic ovum pick up. Cloning Stem Cells. 5:279-285.

Baldassarre, H., K.M. Rao, N. Neveu, E. Brochu, I. Begin, E. Behboodi and D.K. Hockley. 2007. Laparoscopic ovum pick-up followed by *in vitro* embryo production for the reproductive rescue of aged goats of high genetic value. Reproduction, Fertility and Development. 19:612-616.

Bari, F., M. Khalid, W. Haresign, A. Murray and B. Merrell. 2003. Factors affecting the survival of sheep embryos after transfer within a MOET program. Theriogenology. 59:1265-1275.

Baril, G. 1995. Possibilidades atuais da transferencia de embrioes em caprinos. Proceeding of the XI Cong Brasileiro de Reproducao Animal, Belo Horizonte, Brail, 110-120.

Baril, G. and J.C. Vallet. 1990. Time of ovulations in dairy goats induced to superovulate with porcine follicle stimulating hormone during and out of the breeding season. Theriogenology. 34:303-311.

Baril, B., P. Casamitjana, J. Perrin and J.C. Vallet. 1989. Embryo production, freezing and transfer in Angora, Alpine and Saanen goats. Zuchthyg. 24:101-115.

Baril, G., B. Remmy, B. Lebouef, J.C. Vallet, J.F. Beckers and J. Saumande. 1992. Comparison of porcine FSH, caprine FSH and ovine FSH to induce repeated superovulation in goats. Proceeding of the 8th Scientific Meeting of European Embryo Transfer Association, Lyon, France. pp.126 (Abstract).

Baril, G., B. Leboeuf and J. Saumande. 1993a. Synchronization of estrus in goats: the relationship between time of occurrence of estrus and fertility following artificial insemination. Theriogenology. 40:621-628.

Baril, G., P. Brebion and P. Chesne, 1993b. Training Manual for Embryo Transfer in Sheep and Goats. FAO, Rome, Italy. pp: 115 (Abstract).

Baril, G., J.L. Pugmark, V.J.F. Ferias, B. Lebo and J. Summand. 1996. A new method for controlling the precise time of occurance of the preovulatory gonadotropin surge in superovulated goats. Theriogenology. 45:697-706.

Baril, G., A-L. Traldi, Y. Cognie, B. Leboeuf, J.F. Beckers and P. Mermillod. 2001. Successful direct transfer of vitrified sheep embryos. Theriogenology. 56:299-305.

Bartlewski, P.M., A.P. Beard, S.J. Cook and N.C. Rawlings. 1998. Ovarian follicular dynamics during anoestrus in ewes. The Journal of Reproduction and Fertility. 113:275–285.

Bartlewski, P.M., A.P. Beard, S.J. Cook, R.K. Chandolia, A. Hanaramooz and N.C. Rawlings. 1999. Ovarian antral follicular dynamics and their relationships with endocrine variables throughout the oestrous cycle in breeds of sheep differing in prolificacy. The Journal of Reproduction and Fertility. 115:111-124.

Bavister, B.D., C. Dees and R.D. Schultz. 1986. Refractoriness of rhesus monkeys to repeated ovarian stimulation by exogenous gonadotropins is caused by nonprecipitating antibodies. American Journal of Reproductive Immunology and Microbiology. 11:11-16.

Beckers, J.F., G. Baril, J.C. Vallet, D. Chupin, B. Remy and J. Saumande. 1990. Are porcine follicle stimulating hormone antibodies associated with decreased superovulatory response in goat? Theriogenology. 33:192 (Abstract).

Besenfelder, U., N. Zinovieva, E. Dietrich, B. Sohnrey, W. Holtz and G. Brem. 1994. Tubal transfer of goat embryos using endoscopy. Veterinary Record. 135:480-481.

Bessoudo, E., L. Davis, S. Coonrod and D.C. Kraemer. 1988. Commercial embryo transfer in Australian Angora. Theriogenology. 29: 222 (Abstract).

Betteridge, K.J. 1981. An historical look at embryo transfer. The Journal of Reproduction and Fertility. 62:1-13.

Biggers, J.D. and H. Walter. 1991. FRS: a pioneer in reproductive biology. Centenary of his embryo transfer experiments. The Journal of Reproduction and Fertility. 93:173–186.

Bindon, B.M. 1971. Systematic study of preimplantation stages of pregnancy in the sheep. Australian Journal of Biological Sciences. 24: 131-147.

Bindon, B.M., L.R. Piper, L.P. Cahill, M.A. Driancourt and T. O'Shea. 1986. Genetic and hormonal factors affecting superovulation. Theriogenology. 25:53-70.

Bittman, E.L., A.H. Kaynard, D.H. Olster, J.E. Robinson, S.M. Yellon and F.J. Karsh. 1985. Pineal melatonin mediates photoperiodic control of pulsatile luteinizing hormone secretion in the ewe. Neuroendocrinology. 40:409–418.

Brebion, P., G. Baril, Y. Cognie and J.C. Vallet. 1992. Embryo transfer in sheep and goats. Annales De Zootechnie. 41:331-339.

Breuel, K.F., R.D. Baker, R.L. Buther, E.C. Townsend, E.K. Inskeep, R.A. Dailey and S.P. Lerner. 1991. The effects of breed, age of the donor and dosage of follicle stimulating hormone on the superovulatory response of beef-cows. Theriogenology. 36:241-255.

Boland, M.P., F. Lemainque and I. Gordon. 1978. Comparison of lambing outcome in ewes after synchronization of estrus by progestagen or prostaglandin treatment. The Journal of Agricultural Science. 91:765-766.

Boland, M.P., P. Lonergan and D. O'Callaghan. 2001. Effect of nutrition on endocrine parameters, ovarian physiology, and oocyte and embryo development. Theriogenology. 55:1323-1340.

Bondurant, R.H., S. Skirrow, G.B. Anderson, F. Hanson and W.H. Rogers. 1984. Nonsurgical collection of blastocysts from dairy goats. Theriogenology. 22:423- 431.

Cahill, L.P and P. Mauleon. 1980. Influences of season, cycle and breed on follicular growth rates in sheep. The Journal of Reproduction and Fertility. 58:321–328.

Cahill, L.P., J.C. Mariana and P. Mauleon. 1979. Total ovarian follicular populations in ewes of high and low ovulation rates. The Journal of Reproduction and Fertility. 55:27-36.

Cameron, A.W.N., K.M. Battye and A.O. Trounson. 1988. Time of ovulation in goats (*Capra hircus*) induced to superovulate with PMSG. The Journal of Reproduction and Fertility. 83:747-752

Campbell, B.K., A.S. McNeilly, G.E. Mann and D.T. Baird. 1991. The effect of stage of estrous cycle and follicular maturation on ovarian inhibin production in sheep. Biology Reproduction. 44:483-490.

Carnevale, E.M., D.R. Bergfelt and O.J. Ginther**.** 1993. Aging effects on follicular activity and concentrations of FSH, LH, and progesterone in mares. Animal Reproduction Science. 31(3-4):287-299.

Carnevale, E.M., M.J. Hermenet and O.J. Ginther**.** 1997. Age and pasture effects on vernal transition in mares. Theriogenology. 47(5):1009-1018.

Chang, M.C. 1950. Development and fate of transferred rabbit ova and blastocysts in relation to the ovulation time of recipients. Journal of Experiment Zoology. 114:197–226.

Chemineau, P., R. Procureur, Y. Cognie, P.C. Lefevre, A. Locatelli and D. Chupin. 1986. Production, freezing and transfer of embryos from a bluetongue-infected goat herd without bluetongue transmission. Theriogenology. 26(3):279-290.

Chemineau, P., G. Ba, B. Leboeuf, M.C. Maurel, F. Roy, M. Pellicer-Rubio, B. Malpaux and Y. Cognie. 1999. Implications of recent advances in reproductive physiology for reproductive management of goats. The Journal of Reproduction and Fertility. 54:129-142.

Chemineau, P., A. Daveau and Y. Cognie. 2004. Seasonal ovulatory activity exists in tropical female goats and Black Belly ewes subjected to a temperate photoperiod. BMC Physiology. 27:12.

Chupin, D. and J. Saumande. 1979. New attempts to decrease the variability of ovarian response to PMSG in cattle. Annales de Bioligie Animale, Biochimie et Biophysique. 19:1489-1498.

Coffey, L., H. Margo and W. Ann. 2004. Goats: Sustainable Production Overview. Retrieved 26 June 2010. http://ucce.ucdavis.edu/files/filelibrary/1808/25475.pdf

Cognie, Y. 1999. State of art in sheep-goat embryo transfer. Theriogenology. 51:105-116.

Cognie,Y., G. Baril, N. Poulin and P. Mermillod. 2003. Current status of embryo technologies in sheep and goat. Theriogenology. 59(1):171-188.

Critser, J.K., F.C. Gunsett and R.P. Winch. 1979. Factors affecting ova transfer in Limousin, Maine-anjou and Simmental cattle. Theriogenology. 11(1):95.

Curnock, R.M., B.N. Day and P.J. Dziuk. 1975. Embryo transfer in pigs: a method for introducing genetic material into primary specific-pathogen-free herds. American Journal of Veterinary Research. 37:97–98.

De Castro, T., E. Rubianes, A. Menchaca and A. Rivero. 1999. Ovarian dynamics, serum estradiol and progesterone concentrations during the interovulatory interval in goats. Theriogenology. 52: 399-411.

Demoustier, M.M., J.F. Beckers, P.V.D. Zwalmen, J. Closset, J.L. Gillard and F. Ectors. 1988. Determination of porcine plasma folltropin levels during superovulation treatment in cows. Theriogenology. 30:379-386.

Dobbs, K.E., D.A. Dumesic, J.A. Dumesic and S.S. Shapiro. 1994. Differences in serum follicle stimulating hormone uptake after intramuscular and subcutaneous human menopausal gonadotropin injection. Fertility and Sterility. 62:978-983.

Donaldson, L.E. 1984. Cattle breed as source of variation in embryo transfer. Theriogenology. 21:1013-1018.

Driancourt, M.A. and M. Avdi. 1993. Effect of the physiological stage of the ewe on the number of follicles ovulating following hCG injection. Animal Reproduction Science. 32: 227-236.

Driancourt, M.A., R.C. Fry, B.K. Campbell and A.S. McNeilly. 1990. Granulosa cell content and production of steroids, inhibin and follicular peptides by large follicles from a range of prolific and non-prolific breeds of sheep. The Journal of Reproduction and Fertility. 43:230-231.

Drion, P.V., V. Furtoss, G. Baril, E. Manfredi, F. Bouvier, J. Pougnard, D. Bernelas, P. Caugnon, E.M. McNamara, B. Remy, J. Sulon, J. Beckers, L. Bodin and B. Lebceuf. 2001. Four years of induction/synchronization of estrus in dairy goats: effect on the evolution of eCG binding rate in relation with the parameters of reproduction. Reproduction Nutrition Development. 41:401-412.

Dufour, J.J., Y. Cognie, P. Mermillod, J.C. Mariana and R.F. Romain. 2000. Effects of the Booroola *Fec* gene on ovarian follicular populations in the superovulated Romanov ewes pretreated with GnRH antagonist. The Journal of Reproduction and Fertility. 118:85-94.

Dziuk, P.J., J.D. Donker. J.R. Nichols and W.E. Petersen. 1958. Problems associated with the transfer of ova between cattle. Minnesota Agriculture Experiment Station Bulletin. pp. 222.

Eiamvitayakorn, J., N.G. Natural and C.L. Apelo. 1988. Superovualtion treatment in goats (*Capra hircus*). The Thai Journal of Veterinary Medicine. 18:251-258.

El-Gayar, M. and W. Holtz. 2001. Vitrification of goat embryos by the open pulled-straw method. Journal of Animal Science. 79:2436-2438.

Evans, G. and D.T. Armstrong. 1984. Reduction of sperm transport in ewes by superovulation treatments. The Journal of Reproduction and Fertility. 70:47-53.

Evans, A.C., J.D. Flynn, P. Duffy, P.G. Knight and M.P. Boland. 2002. Effects of ovarian follicle ablation on FSH, oestradiol and inhibin A concentrations and growth of other follicles in sheep. Reproduction. 123:59–66.

Fatet, A., M.T. Pellicer-Rubio and B. Leboeuf. 2011**.** Reproductive cycle of goat. Animal Reproduction Science. 124(3-4):211-219.

Flores-Foxworth, G., B.M. McBride, D.C. Kr Hawk Aemer and L.C. Nuti. 1992. A comparison between laparascopic and transcervical embryo collection and transfer in goats. Theriogenology. 37:213 (Abstract).

Forcada, F., M.A. Amer-Meziane, J.A. Abecia, M.C. Maurel, J.A. Cebrian-Perez, T. Muino-Blanco, B. Asenjo, M.I. Vazquez and A. Casao. 2010. Repeated superovulation using a simplified FSH/eCG treatment for *in vivo* embryo production in sheep. Theriogenology. 75(4):769-776.

Fuki, Y., H. Kano, M. Kobayashi, M. Tetsura and H. Ono. 1985. Response to repeated superovulation treatment in the ewe. Japanese Journal of Animal Reproduction. 31:155-157.

Gibbons, J.R., K. Kot, D.L. Thomas, M.C. Wiltbank and O.J. Ginther. 1999. Follicular and FSH dynamics in ewes with a history of high and low ovulation rates. Theriogenology. 52:1005–1020.

Gibbons, A., B.F. Pereyra, M.I. Cueto, M. Catala, D.F. Salamone and A. Gonzalez-Bulnes. 2007. Procedure for maximizing oocyte harvest for in vitro embryo production in small ruminants. Reproduction on Domestic Animal. 42(4):423-6.

Ginther, O.J. and K. Kot. 1994. Follicular dynamics during the ovulatory season in goats. Theriogenology. 42:987-1001.

Ginther, O.J., M.A. Beg, D.R. Bergfelt, F.X. Donadeu and K. Kot. 2001. Follicle selection in monovular species. Biology Reproduction. 65:638–647.

Godfrey, R.W., J.R. Collins, E.L. Hensley and J.E. Wheaton. 1999. Oestrous synchronisation and artificial insemination of hair sheep ewe in the tropics. Theriogenology. 51:985–997.

Goel, A.K. and K.P. Agrawal. 1990. Superovulation and embryo collection in Jamunapari goats. Theriogenology. 33(1):232.

Gonzalez-Bulnes, A., J. Santiago-Moreno, A. Gomez-Brunet, E.K. Inskeep, E.M. Townsend and A. Lopez-Sebastian. 1999. Follicular dynamics during the oestrous cycle in dairy goats. Animal Science. 68:547-554.

Gonzalez-Bulnes, A., R.M. Garcia-Garcia, J. Santiago-Moreno, A. Lopez-Sebastian and M.J. Cocero. 2002. Effect of follicular status on superovulatory response in ewes is influenced by presence of corpus luteum at first FSH dose. Theriogenology. 58:1607-1614.

Gonzalez-Bulnes, A., R.M. Garcia-Garcia, J. Santiago-Moreno, V. Dominguez, A. Lopez-Sebastian and M.J. Cocero. 2003. Reproductive season affects inhibitory effects from large follicles on the response to superovulatory FSH treatments in ewes. Theriogenology. 60:281-288.

Gonzalez-Bulnes, A., D.T. Baird, B.K. Campbell, M.J. Cocero, R.M. Garcia-Garcia, E.K. Inskeep, A. Lopez-Sebastian, A.S. McNeilly, J. Santiago-Moreno, C.J.H. Souza and A. Veiga-Lopez. 2004. Multiple factors affecting efficiency of multiple ovulation and embryo transfer in sheep and goats. Reproduction, Fertility and Development. 16:421-435.

Gordon, I. 1997. Controlled reproduction in sheep and goats. Centre for Agricultural Bioscience International, U.K.

Greyling, J.P.C. and C.H.V. Niekerk. 1987. Occurrence of oestrus in the Boer goat doe. South Africa Journal Animal Science. 17:147-149.

Greyling, J.P.C. and M.V.D. Nest. 2000. Synchronization of oestrous in goats: dose effect of progestagen. Small Ruminant Research. 36:201-207.

Greyling, J.P.C., M.V.D. Nest, L.M.J. Schwalbach and T. Muller. 2002. Superovulation and embryo transfer in South African Boer and indigenous feral goats. Small Ruminant Research. 43:45-51

Guignot, F., A. Bouttier, G. Baril, P. Salvetti, P. Pignon, J.F. Beckers, J.L. Touze. J. Cognie, A.S. Traldi, Y. Cognie and P. Mermillod. 2006. Improved vitrification method allowing direct transfer of goat embryos. Theriogenology. 66:1004-1011.

Hamra, A.H., J.W. McNally, J.M. Marcek, K.M. Carlson and J.E. Wheaton. 1989. Comparison of progesterone sponges,cronolone sponges and controlled internal drug release dispenserson fertility in anestrous ewes. Animal Reproduction Science. 18:219-226.

Hasler, J.F. 1992. Current status and potential of embryo transfer and reproductive technology in dairy cattle. Journal of Dairy Science. 75: 2857-2879.

Heap, R.B. 1992. Mr. Walter Heape M.A., F.R.S. (1855–1929). In: Proceedings of the Eighth Meeting of the European Embryo Transfer Association, Lyon, September 11–12, pp. 109–124.

Heape, W. 1891. Preliminary note on the transplantation and growth of mammalian ova within a uterine foster-mother. Proceedings of the Roy Society Biological Science 48:457–458.

Herve, V., F. Roy, J. Bertin, F. Guillou and M. Maurel. 2004. Antiequine chorionic gonadotropin (eCG) antibodies generated in goats treated with eCG for induction of ovulation modulate the luteinizing hormone and follicle-stimulating hormone boiactivities of eCG differently. Endocrinology. 145:294-303.

Hirst, K.K. 2008. The History of the Domestication of Goats. Retrieved 18 August 2009. http://archaeology.about.com/od/domestications/qt/goats.htm.

Holcomb, G.B. 1994. A small-Scale agricultural alternative: dairy and eat goats. USDA cooperative state research service, the office for small-scale agriculture, Washington, DC. 2 p

Holtz, W. 1996. Embryotransfer bei der Ziege-eine UE bersicht. Dtsch. TieraErztl. Wochenschr. 103:293-297.

Holtz, W. 2005. Recent developments in assisted reproduction in goats. Small Ruminant Research. 60:95-110.

Hunter, R.H.F. and I. Wilmut. 1983. The rate of functional sperm transport into the oviducts of mated cows. Animal Reproduction Science. 5:167-173.

Ishwar, A.K. and M.A. Memon. 1996. Embryo transfer in sheep and goats: a review. Small Ruminant Research. 19:35-43.

Jainudeen, H.R., H. Wahid and E.S.E. Hafez. 2000. Sheep and goats. Proceeding: Reproduction in Farm Animals. 7th Edition. Editors: E.S.E. Hafez, B. Hafez. Lippincott Williams and Wilkins, Philadelphia, USA.

Kafi, M. and M.R. McGowan. 1997. Factors associated with variation in the superovulatory response of cattle. Animal Reproduction Science. 48:137-157.

Kaneko, H., Y. Nakanishi, S. Akagi, K. Arai, K. Taya and G. Watanabe. 1995. Immunoneutralization of inhibin and estradiol during the follicular phase of the estrous cycle in cows. Biology of Reproduction. 53:931–939.

Kaneko, H., J. Noguchi, K. Kikuchi, J. Todoroki and Y. Hasegawa. 2002 Alterations in peripheral concentrations of inhibin A in cattle studied using a time-resolved immunofluorometric assay: relationship with estradiol and follicle-stimulating hormone in various reproductive conditions. Biology of Reproduction. 67:38–45.

Kawate, N., N. Morita, M. Tsuji, H. Tamada, T. Inaba and T. Sawada. 2000. Roles of pulsatile release of LH in the development and maintenance of corpus luteum function in the goat. Theriogenology. 54:1133-1143.

Keskintepe. L., G.C. Luvoni, S.J. Rzucidlo and B.G. Brackett. 1996. Procedural improvements for in vitro production of viable uterine stage caprine embryos. 20(3):247-254.

Kiessling, A.A., W.H. Hughes and M. R. Blankevoort. 1986. Superovulation and embryo transfer in the dairy goat. Journal of the American Veterinary Medical Association. 188:829-832.

Kuhholzer, B. and G. Brem. 1999. In vivo development of micro injected embryos from superovulated prepubertal slaughter lambs. Theriogenology. 51:1297- 1302.

Kumar, J., J.C. Osborn, A.W.N. Cameron, P.A. Batt and A.O. Trounson. 1990. Premature condensation of chromatin induced in goat (*Capra hircus*) oocytes after gonadotrophin treatment. Reproduction, Fertility and Development. 2:661-670.

Kumar, J., J.C. Osborn and A.W.N. Cameron. 1991. Luteinizing hormone and follicle stimulating hormone induce premature condensation of chromatin in goat (*Capra hircus*) oocytes. Reproduction, Fertility and Development. 3:585-591.

Kvasnitski. A.V. 1950. The research on interbreed ova transfer in pigs. Socialist Livestock Breeding Journal, Semi-annual Report of the Ukrainian Ministry of Agriculture. pp. 12-15.

Leboeuf, B., E. Manfredi, P. Boue, A. Peacere, G. Brice, G. Baril, C. Broqua, P. Humblot and M. Terqui. 1998. Artificial insemination of dairy goats in France. Livestock Production Science. 5:193–203.

Lehloenya, K.C. 2008. Multiple ovulation and embryo transfer in goats. Ph.D. Dissertation. University of the Free State Bloemfontein.

Lehloenya, K.C. and J.P.C. Greyling. 2009. Effect of route of superovulatory gonadotrophin administration on the embryorecovery rate of Boer goat does. Small Ruminant Research. 87(1-3):49-44.

Lehloenya, K.C. and J.P.C. Greyling. 2010. The ovarian response and embryo recovery rate in Boer goat does following different superovulation protocols, during the breeding season. Small Ruminant Research. 88(1):38-43.

Lehloenya, K.C., J.P.C. Greyling and L.M.J. Schwalbach. 2005. Reproductive performance of South African indigenous goats following oestrous synchronization and A.I. Small Ruminant Research. 57:115-120.

Lehloenya, K.C., J.P.C. Greyling and S. Grobler. 2008. Effect of season on the superovulatory response in Boer goat does. Small Ruminant Research. 78(1-3):74-79.

Li, R., A.W.N. Cameron, P.A. Batt and A.O. Trounson. 1990. Maximum survival of frozen goat embryos is attained at the expanded, hatching and hatched blastocyst stages of development. Reproduction, Fertility and Development. 2:345-350.

Lincoln, G.A. 2006. Decoding the nightly melatonin signal through circadian clockwork. Molecular and Cellular Endocrinology. 252(1-2):69-73.

Mahmood, S., G.L. Koul and J.C. Biswas. 1991. Comparative efficacy of FSH-P and PMSG on superovulation in Pashmina goats. Theriogenology. 35:1191-1196.

Mani, A.U., W.A.C. McKelvey and E.D. Watson. 1992. The effects of low level of feeding on response to synchronization of estrus, ovulation rate and embryo loss in goats. Theriogenology. 38:1013-1022.

Mann, G.E., A.S. McNeilly and D.T. Baird. 1992. Hormone production in vivo and in vitro from follicles at different stages of the estrous cycle in sheep. Journal of Endocrinology. 132:225–234.

McEvoy, T.G., J.J. Robinson, R.P. Aitken, P.A. Findlay and I.S. Robertson. 1997. Dietary excesses of urea influence the viability and metabolism of preimplantation sheep embryos and may affect fetal growth among survivors. Animal Reproduction Science. 47:71-90.

McKelvey, W.A.C., J.J. Robinson and R.P. Aitken. 1985. A simplified technique for the transfer of ovine embryos by laparoscopy. Veterinary Record. 117:492-494.

McMillan.W.H. and D.R.H. Hall. 1994. Laparoscopic transfer of ovine and cervine embryos using the transpic technique. Theriogenology. 42(1):137-146.

Medan, M.S., G. Watanable, K. Sasaki, S. Sharawy, N.P. Groome and K. Taya. 2003. Ovarian dynamics and their associations with peripheral concentrations of gonadotrophins, ovarian steroids and inhibin during the estrous cycle in goats. Biology Reproduction. 69:57-63.

Menchaca, A. and E. Rubianes. 2002. Relation between progesterone concentrations during the early luteal phase and follicular dynamic in goats. Theriogenology. 57:1411-1419.

Mitchell, L.M., W.S. Dingwall, M.J.A. Mylne, J. Hunton, K. Matthews, F.E. Gebbie, G. J. McCallum and T.G. McEvoy. 2002. Season affects characteristics of the pre-ovulatory LH surge and embryo viability in superovulated ewes. Animal Reproduction Science. 74:163-174.

Monniaux, D., D. Chupin and J. Saumande. 1983. Superovulatory responses of cattle. Theriogenology. 19:55-81.

Moor, R.M., Th.A.M. Kruip and D. Green. 1984. Intraovarian control of folliculogenesis: limits to superovulation. Theriogenology. 21:103-116.

Moore, N.W. 1974. Multiple ovulation and ovum transfer in the goat. Proceedings of the Australian Society of Animal Production. 10:246-249.

Moore, N.W. 1982. Egg transfer in the sheep and goat. In Adams CF Ed, Mammalian Egg Transfer. CRC Press, Boca Raton, Florida. 119-133.

Moore, N.W. and J.N. Shelton. 1964. The response of the ewe to a horse anterior pituitary extract. The Journal of Reproduction and Fertility. 52:145 (Abstract).

Moore, N.W. and J. Eppleston. 1976. The use of embryo transfer in the Angora goat. Theriogenology. 6 (6):638. (Abstract).

Moore, N.W. and J. Eppleston. 1979. Embryo transfer in the Angora goat. Australian Journal of Agricultural Research. 30:973-981.

Morel, M.C.G.D., J.R. Newcombe and J.C. Swindlehurst. 2005. The effect of age on multiple ovulation rates, multiple pregnancy rates and embryonic vesicle diameter in the mare. Theriogenology. 63:2482–2493.

Morel, M.C.G.D., J.R. Newcombe and K. Hayward. 2010. Factors affecting pre-ovulatory follicle diameter in the mare: the effect of mare age, season and presence of other ovulatory follicles (multiple ovulation). Theriogenology. 74(7):1241-1274.

Morris, L.H. and W.R. Allen. 2002. Reproductive efficiency of intensively managed Thoroughbred mares in Newmarket. Equine Veterinary Journal. 34(1):51-60.

Motlomelo, K.C., J.P.C. Greyling and L.M.J. Schwalbach. 2002. Synchronisation of oestrus in goats: the use of different progestagen treatments. Small Ruminant Research. 45:45-49.

Mutter, L.R., A.P. Graden and D. Olds. 1964. Successful non-surgical bovine embryo transfer. A.I. Dig. 12: 3.

Nagashima, H., K. Matsui, T. Sawasaki and Y. Kano. 1987. Nonsurgical collection of embryos in Shiba goats. Experiment Animal. 36:51-56.

Nagy, A., M. Gertsenstein, K. Vintersten and R. Behringer. 2003. Manipulating the Mouse Embryo: A Laboratory Manual. Third edition. Cold Spring Harbour Press, Cold Spring Harbour, New York.

Naqvi, S.M.K. and R. Gulyani. 1998. The effect of gonadotrophin releasing hormone and follicle stimulating hormone in conjunction with pregnant mare serum gonadotrophin on the superovulatory response in crossbred sheep in India. Tropical Animal Health and Production. 30:369-376.

Nellenschulte, E. and H. Niemann. 1992. Collection and transfer of ovine embryos by laparoscopy. Animal Reproduction Science. 27:293-304.

Nicholas, J.S. 1933. Development of transplanted rat eggs. Proceedings of the Society for Experiment Biology and Medicine. 30:1111–1113.

Nolan, R., D. O´Callaghan, R.T. Duby, P. Lonergan and M.P. Boland. 1998. The influence of short-term nutrient changes on follicle growth and embryo production following superovulation in beef heifers. Theriogenology. 50:1263- 1274.

Nowshari, M.A. and W. Holtz, 1993. Transfer of split goat embryos without Zonae Pellucidae either fresh or after freezing. Journal of Animal Science. 71:3403-3408.

Nowshari, M.A., E. Yuswiati, M. Puls-Kleingeld and W. Holtz. 1992. Superovulation in peripubertal and adult goats treated with PMSG or pFSH. In: Recent Advances in Goat Production. Editors: Lokeswhar, R.R. New Delhi. pp: 1358-1363.

Nowshari, M.A., J.F. Beckers and W. Holtz. 1995. Superovulation of goats with purified pFSH supplemented. Theriogenology. 43: 797-802.

Nuti, L.C., B.S. Minhas, W.C. Baker, J.S. Capehart and P. Marrack. 1987. Superovulation and recovery of zygotes from Nubian and Alpine dairy goats. Theriogenology. 28: 481-488.

O'Callaghan D and M. Boland. 1999. Nutritional effects on ovulation, embryo development and the establishment of pregnancy in ruminants. Journal of Animal Science. 68:299-314.

Oguri, N and Y. Tsutsumi. 1974. Non-surgical egg transfer in mares. The Journal of Reproduction and Fertility. 41: 313–320.

Oliveira, M.A.I., S.I. Guido and P.F. Lima. 2001. Comparison of different protocols used to induce and synchronize estrus cycle of Saanen goats. Small Ruminant Research. 40: 149-153.

Pampoukidou, A., T. Alifakiotis, M. Avdi and I. Magras. 1992. Superovulation and embryo transfer in goats by using PMSG or FSH. Proceeding of the 8th Meeting of European Embryo Transfer Association, 198.

Pargaonkar, M.D., S.A. Bakshi, D.R. Pargaonkar, M.K. Tandle and S.V. Doijode. 1994. Studies on superovulation response of goats treated with PMSG. India Journal of Dairy Science. 47:149-150.

Parr, R.A., I.F. Davis, R.J. Fairclough and M.A. Miles. 1987. Overfeeding during early pregnancy reduces peripheral progesterone concentration and pregnancy rate in sheep. The Journal of Reproduction and Fertility. 80: 317-320.

Pendleton, R.J., C.R. Youngs, R.W. Rorie, M.A. Memon and R.A. Godke. 1986. The use of Norgestomet implants and FSH for estrus synchronization and superovulation in goats. Theriogenology. 25: 180 (Abstract).

Pendleton, R.J., C.R. Youngs, R.W. Rorie, S.H. Pool, M.A. Memon and R.A. Godke. 1992. Follicle stimulating hormone versus pregnant mare serum gonadotropin for superovulation of dairy goats. Small Ruminant Research. 8: 217-224.

Pereira, R.J.T.A., B. Sohnrey and W. Holtz. 1998 Nonsurgical embryo collection in goats treated with prostaglandin F2a and oxytocin. Journal Animal Science. 76: 360-363.

Peura, T.T., D.O. Kleemann, S.R. Rudiger, G.S. Nattrass, C.J. McLaughlan and S.K. Walker. 2003. Effect of nutrition of oocyte donor on the outcomes of somatic cell nuclear transfer in sheep. Biology of Reproduction. 68: 45-50.

Pevet, P., L. Agez, B. Bothorel, M. Saboureau, F. Gauer, V. Laurent and M. Masson-Pevet. 2006. Melatonin in the multi-oscillatory mammalian circadian world. Chronobiology International. 23(1-2): 39–51.

Phillippo, M. and L.E.A. Rowson. 1975. Prostaglandins and superovulation in the bovine. Annales de Biologie Animale, Biochimie, Biophysique 15: 233–240.

Pinczak, A., A. Menchaca and E. Rubianes. 2001. Seguimiento ultrasonografico ovaricoy uterino durante la gestación temprana de la cabra (Ovarian and uterine scanning during the early pregnancy in goats). In: Proceedings of the IV International Symposium on Animal Reproduction. Cordoba, Argentina, p. 298 (abstract).

Pintado. B., A. Gutierrez-Adan**,** B. Perez and J. Garde**.** 1997. Influence of the synchronization treatment on the superovulatory response of Murciana goats. Small Ruminant Research. 23(2-3):135-141.

Pintado, B., A. Gutierrez-Adan and B. Perez Llano. 1998. Superovulatory response of Murciana goats to treatments based on PMSG/anti-PMSG or combined FSH/PMSG administration. Theriogenology. 50: 357-364.

Prather, R.S., F.L. Barnes, M.M. Sims, J.M. Robl, W.H. Eyestone and N.L. First. 1987. Nuclear transplantation in the bovine embryo: assessment of donor nuclei and recipient oocyte. Biology of Reproduction. 37: 859–866.

Polejaeva. I.A. and K.H.S. Campbell**.** 2000. New advances in somatic cell nuclear transfer: application in transgenesis. Theriogenology. 53(1):117-126.

Polge, C. and B.N. Day. 1968. Pregnancy following nonsurgical egg transfer in pigs. Veterinary Record. 82: 712 (abstract).

Quirke, J.F. and J. P. Hanrahan. 1977. Comparison of the survival in the uteri of adult ewes of cleaved ova from adult ewes and ewe lambs. The Journal of Reproduction and Fertility. 51: 487-489.

Rangel-Santos, R., M.F. McDonald and G.A. Wickham. 1991. Evaluation of the feasibility of a juvenile MOET scheme in sheep. New Zealand Society of Animal Production. 51: 139-142.

Remy, B., G. Baril, J.C. Vallet, R. Dufour, C. Chouvet, J. Saumande, D. Chupin and J.F. Beckers. 1991. Are antibodies responsible for a decreased superovulatory response in goats which have been treated repeatedly with porcine follicle-stimulating hormone? Theriogenology. 36: 389-399.

Restall, B.J. 1992. Seasonal variation in reproductive activity in Australian goats. Animal Reproduction Science. 27(4):305-318.

Rhodes, F.M., G. De'ath and K.W. Entwistle. 1995. Animal and temporal effects on ovarian follicular dynamics in Brahman heifers. Animal Reproduction Science. 38(4):265-277.

Riesenburg, S., S. Meinecke-Tillmann and B. Meinecke. 2001. Ultrasonic survey of follicular development following superovulation with single application of pFSH, eCG or hCG in goats. Small Ruminant Research. 40: 83-93.

Rosnina, Y., M.R. Jainudeen and M. Nihayah. 1992. Superovulation and egg recovery in goats in the tropics. Veterinary Record. 130: 97-99.

Roy, F., M. Maurel, B. Combes, D. Vaiman, E.P. Cribiu, I. Lantier, T. Pobel, F. Deletang, Y. Combarnous and F. Guillou. 1999. The negative effect of repeated equine chorionic gonadotrophin treatment on subsequent fertility in Alpine goats is due to a humoral immune response involving the major histocompatibility complex. Biology of Reproduction. 60: 805-813.

Rowson, L.E.A. 1971. Egg transfer in domestic animals. Nature. 233: 379-381.

Rubianes, E. and A. Menchaca. 2003. The pattern and manipulation of ovarian follicular growth in goats. Animal Reproduction Science. 78:271-87.

Rubianes, E., R. Ungerfeld and D. Ibarra. 1996. Use of serum anti-eCG improve luteal function and increase ova/embryos recovery in eCG-superovulated ewes. Small Ruminant Research. 21: 105–111.

Saharrea, A., J. Valencia, A. Balcazar, O. Meija, J.L. Cerbon, V. Caballero and L. Zarco. 1998. Premature luteal regression in goats superovulated with PMSG: effect of hCG or GnRH administration during the early luteal phase. Theriogenology. 50:1039-1052.

Sahlu, T. and A.L. Gvestsch. 2005. A foresight on goat research. Small Ruminant Research. 60 (1-2):7-12.

Saumande, J., R. Procureur and D. Chupin. 1984. Effect of injection time of anti- PMSG antiserum on ovulation rate and quality of embryos in superovulated cows. Theriogenology. 21: 727-731.

Scaramuzzi, R.J. and J.F. Murray. 1994. The nutrient requirements for the optimum production of gametes in assisted reproduction in ruminant animals. 12th Association Europeenne de Transfer Embryonnaire. pp. 85-103.

Scaramuzzi, R.J., B.K. Campbell, J.A. Downing, N.R. Kendall, M. Khalid, M. Munoz-Gutierrez and A. Somchit. 2006. A review of the effects of supplementary nutrition in the ewe on the concentrations of reproductive and metabolic hormones and the mechanisms that regulate folliculogenesis and ovulation rate. Reproduction Nutrition Development. 46: 339-354.

Schwarz, T. and E. Wierzchos. 2000. Relationship between FSH and ovarian follicular dynamics in goats during the estrous cycle. Theriogenology. 53: 381.

Sebastian-Lopez, A., Y. Cognie, M.J. Cocero, J.D.L. Fuente and N. Poulin. 1990. Effect of season and duration of FSHp treatment on embryo production in sheep. Theriogenology 34: 175-180.

Selgrath, J.P., M.A. Memon, T.E. Smith and K.M. Ebert. 1990. Collection and transfer of microinjectable embryos from dairy goats. Theriogenology. 34: 1195- 1205.

Senn, B.J. and M.E. Richardson. 1992. Seasonal effects on caprine response to synchronization of estrus and superovulatory treatment. Theriogenology. 37: 579-585.

Senthil, K.P., D. Saravanan, R.C. Rajasundaram, M. Selvaraju and D. Kathiresan. 2003. Serum oestradiol and progesterone profiles and their relationship with superovulatory responses in Tellicherry goats treated with eCG and FSH. Small Ruminant Research. 49: 69-77.

Shamsul, A. A. S. 1997. Effects of Superovulation Regimes on steroid hormones and embryo production for laparoscopic embryo transfer programme in goats. M.Phil. Dissertation. University of Malaya, Kuala Lumpur, Malaysia.

Shariffah, N. 2012. Effect of superovulation on ovarian responses and subsequent embryo transfer in goats. M.Sc. Dissertation. University of Malaya, Kuala Lumpur, Malaysia.

Simoes, J., J. Potes, J. Azevedo, J.C. Almeida, P. Fontes, G. Baril and R. Mascarenhas. 2005. Morphometry of ovarian structures by transrectal ultrasonography in Serrana goats. Animal Reproduction Science. 85: 263–273.

Sirard, M.A., F. Richard, P. Blondin and C. Robert. 2006. Contribution of the oocyte to embryo quality. Theriogenology. 65(1):126-136.

Smith, J.F. 1976. Selection for fertility and response to PMSG in Romney ewes selection. New Zealand Society of Animal Production. 36: 247-251.

Smith, J.F. 1991. A review of recent developments on the effect of nutrition on ovulation rate (the flushing effect) with particular reference to research at Ruakura. New Zealand Society of Animal Production. 51: 15-23.

Smith, A.K. and S.P. Grimmer. 2002. Pregnancy rates for Grade 2 embryos following administration of synthetic GnRH at the time of transfer in embryo-recipient cattle. Theriogenology. 57:2083-2091.

Sohnrey, B.S. and W. Holtz**.** 2000. Transcervical embryo collection in Boer goats. Small Ruminant Research. 36(2):195-200.

Spearow, J. L. 1988. Characterization of genetic differences in hormone-induced ovulation rate in mice. Journal of Reproduction and Fertility. 82:799-806.

Steptoe, P.C. and R.G. Edwards. 1978. Birth after the reimplantation of a human embryo. The Lancet ii: 366.

Stice, S.L. and J.M. Robl. 1988. Nuclear reprogramming in nuclear transplant rabbit embryos. Biology of Reproduction. 39:657–664.

Suyadi, B., W. Sohnrey and W. Holtz. 2000. Transcervical embryo collection in Boer goats. Small Ruminant Research 36: 195-200.

Suyadi, W.X., S. Wallenhorst, J.F. Beckers and W. Holtz. 2005. Endocrine response, ovulation rate and embryo yield in goats superovulated with different pFSH treatment regimes. (in preparation)

Tervit, H.R., P.G. Goold and R.D. McKenzie. 1984. Embryo transfer in Angora and Saanen goats. Theriogenology. 21 (1):269 (Abstract).

Tervit, H.R., P.G. Goold and R.D. McKenzie. 1986. Development of an effective goat embryo transfer regime. New Zealand Society of Animal Production. 46: 233-236.
Torres, S., Y. Cognie and G. Colas. 1987. Transfer of superovulated sheep embryos obtained with different FSH-P. Theriogenology. 27: 407-419.

Tritschler, J.P., R.T. Duby, E.M. Parsons, M.J. Parsons and D.J. Giordano. 1991. Comparison of two progestogens during out-of-season breeding in a commercial ewe flock. Theriogenology. 35:943-952.

Tsunoda, Y. and T. Sugie. 1989. Superovulation in nonseasonal Japanese native goat, with special reference to the developmental progression of embryo. Theriogenology. 31: 991-996.

Umbaugh, R.E. 1949. Superovulation and ovum transfer in cattle. American Journal of Veterinary Research. 10: 295–305.

Ungerfeld, R. and E. Rubianes. 1999. Oestrus response to the ram effect in Corriedale ewes primed with medroxyprogesterone during the breeding season. Small Ruminant Research. 32(1):89-91.

Viñoles, C., M. Forsberg, G. Banchero and E. Rubianes. 2002. Ovarian follicular dynamics and endocrine profiles in Polwarth ewes in high and low body condition. Animal Science. 74: 539–545.

Vivanco, H.M., K.B. Greany and H. Varela. 1994. Explaining the variability in superovulatory responses and yield of transferable embryos in sheep embryo transfer. Theriogenology. 41: 329 (Abstract).

Walker, S.K., D.H. Smith, A. Frensham, R.J. Ashman and R.F. Seamark. 1989. The use of synthetic gonadotropin releasing hormone treatment in the collection of sheep embryos. Theriogenology. 31:741-753.

Wallace, J. M. 1992. Artificial insemination and embryo transfer. In: Progress in sheep and goat research. Editors: Speedy, A.W. CAB International, U.K.

Walters, D.L., W.C. Burrell and J.N. Wiltbank. 1984. Influence of exogenous steroids, nutrition and calf removal on reproductive performance of anestrous beef cows. Theriogenology. 21(3):395-406.

Wang, B., H. Baldassarre, J. Pierson, F. Cote, K.M. Rao and C.N. Karatzas. 2003. The in vitro and *in vivo* development of goat embryos produced by intracytoplasmic sperm injection using tail-cut spermatozoa. Zygote. 11: 219-227.

Warwick, B.L. and R.O. Berry. 1949. Inter-generic and intra-specific embryo transfers in sheep and goats. Journal of Heredity. 40: 297–303.

Warwick, B.L., R.O. Berry and W.R. Horlacher. 1934. Results of mating rams to Angora female goats. Proceedings of the 27th Annual Meeting of the American Society of Animal Production. pp. 225–227.

Wells, D.N., P.M. Misica and H.R. Tervit. 1998. Production of Cloned Calves Following Nuclear Transfer with Cultured Adult Mural Granulosa Cells. Biology of Reproduction. 60(4):996-105.

Wheaton, J.E., K.M. Carlson, H.F. Windels and L.J. Johnston. 1993. CIDR: a new progesterone-releasing intravaginal device for induction of oestrus and cycle control in sheep and goat. Animal Reproduction Science. 33:127–141.

Wheeler, A.G and R.B. Land. 1977. Seasonal variation in oestrus and ovarian activity of Finnish Landrace, Tasmanian Merino and Scottish Blackface ewes. Animal Production. 24: 363–376.

Whittingham, D.G. and W.K. Whitten. 1974. Long-term storage and aerial transport of frozen mouse embryos. The Journal of Reproduction and Fertility. 36: 433–435.

Wildeus, S. 2004. Current concepts in synchronization of estrus: sheep and goats. Journal of Animal Science. 77: 1-14.

Willadsen, S.M. 1986. Nuclear transplantation in sheep embryos. Nature. 320: 63–65.

Willett, E.L. and P.J. Buckner. 1953. Refractoriness of cows repeatedly superovulated with gonadotrophins. Journal of Dairy Science. 36: 1083-1088.

Wilmut, I. and L.E.A. Rowson. 1973. Experiments on the low-temperature preservation of cow embryos. Veterinary Record. 92: 686–690.

Wilmut, I. and R.H.F. Hunter. 1984. Sperm transport into the oviducts of heifers mated early in oestrus. Reproduction Nutrition Development. 24: 461-468.

Woodruff, T.K, L.M. Besecke, N, Groome, L.B. Draper, N.B. Schwartz and J. Weiss. 1996. Inhibin A and inhibin B are inversely correlated to follicle-stimulating hormone, yet are discordant during the follicular phase of the rat estrous cycle, and inhibin A is expressed in a sexually dimorphic manner. Endocrinology. 137: 5463–5467.

Wrathall, A.E, J.T. Done, P. Stuart, D. Mitchell, K.J. Betteridge and G.C.B. Randall. 1971. Successful intercontinental pig conceptus transfer. Veterinary Record. 87: 226–228.

Wulster-Radcliffe, M.C., B.A. Costine and G.S. Lewis. 1999. Esradiol-17β-oxytocin–induced cervical dilation in sheep: application to transcervical embryo transfer. Journal Animal Science. 77: 2587-2593.

Yamamoto, Y., N. Oguri, Y. Tsutsumi and Y. Hachinohe. 1982. Experiments in the freezing and storage of equine embryos. The Journal of Reproduction and Fertility. 32: 399–403.

Yang, Z.M., J.H. Tan and P.C. Qin. 1991. A preliminary study on the preimplantation development in goats. Acta Veterinary et Zootechnica. Sinica. 22: 32-37.

Yuswiati, E. and W. Holtz. 1990. Successful transfer of vitrified goat embryos. Theriogenology. 34: 629-632.

Zhao, X.C. 2008. In the regulate and control of goat and sheep reproduction. 1st Edition. Editors: Zhao, X.C. Chinese agriculture publishing company. Bei Jing.

APPENDICES

APPENDICES

APPENDIX 1: LIST OF MATERIALS

Appendix Table 1.1: List of equipment and instruments

Equipment/instrument	Model no.	Manufacturer
Atrauatic grasping forcep	PO951R	Aesculap®, Germany
Autoclave	HA-300MII	Hirayama Hiclave, Japan
CIDR applicator	-	Pharmacia and Upjohn, New Zealand
CO_2 incubator,	HeraCell 240	Heraeus, Germany
CO_2 insufflator system	PG001	Aesculap®, Germany
Digital balance	AB104	Mettler Toledo, Switzerland
Dissecting microscope	SZH10	Olympus, Japan
Impulse sealer	KF-300H	Khind, Taiwan
Mobile embryonic laboratory		
Laminar flow cabinet	HLF-120	Gelman Science, Australia
Laparoscopic system:		Aesculap®, Germany
(a) Endoscopic camera system	PV431	
(b) CCD camera	PV430	
(c) Pediatric Storz laparoscope (7 mm)	PE668A	
(d) Light probe with fibre optic cable	OP913	
(e) Light system (300 W)	OP927	
Micropipette dispenser	-	Eppendorf, Gerany
Oven	40050-IP20	Memmert GmbH, Germany
pH meter	HI-122	Hanna Instruments, Singapore
Refrigerator and freezer	SR-21NME	Samsung Electronics, Korea
Stage warmer (Thermoplate)	HATS-U55R30	Tokai Hit, Japan
Stereomicroscope	SZH10	Olypus Optical, Japan
Spirit burner	-	Shanghai Machinery, China
Surgical set	-	Aesculap®, Germany
Surgical table	-	Syarikat Copens Enterprise, Malaysia
Ultrapure purification water system	Milli-Q PF Plus	Millipore, USA
Ultrasound machine	ALOKA SSD500	Leesmed South Korea
Water bath	GMP-GC-19	Meert GmbH, Germany

Appendix Table 1.2: List of chemicals, reagents and media

Cheicals, reagents and media	Catalogue no.	Manufacturer
BME amino acids solution (50x)	B6766	Sigama-Aldrich,USA
Chorulon® (hCG)		Intervet International, Holland
Calcium chloride ($CaCl_2.2H_2O$)	C7902	Sigama-Aldrich,USA
Cloprostenol (Estrumate®)	-	Schering-Plough, Australia
Embryo Hoding Solution	5935882002	EMCARE®, USA
Ethyl alcohol 99.8% (absolute ethanol)	ET150-50	Systerm ChemAR®, Poland
Folligon®		Intervet®, EU
Folltropin®-V	-	Bioniche, Animal Health Canada Inc.
Gentamicin sulphate salt	G3632	Sigama-Aldrich,USA
Goat/sheep pellet feed	-	KMM Berhad, Malaysia
Heparin	H0777	Sigama-Aldrich,USA
Hibiscrub (antiseptic)	HK-06770	SSL International Plc, UK
Intravaginal progesterone release device		Pharmacia and Upjohn, New Zealand
Ketamil injection (ketamine hydrochloride)	L10077	Troy Laboratories, Australia
K-Y Lubricating Jelly	-	Pharmedica Lab, South Africa
L-glutamine	G3126	Sigama-Aldrich,USA
KCl	P5404	Sigama-Aldrich,USA
KH_2PO_4	P5655	
Sodium chloride (NaCl)	S5886	Sigama-Aldrich,USA
$NaHCO_3$	S5761	Sigama-Aldrich,USA
$MgCl_2.6H_2O$	M2393	Sigama-Aldrich,USA
MEM (100x)	M7145	Sigama-Aldrich,USA
PBS Dulbecco A tablets	BR0014G	Oxoid,England
Phenol red solution (0.5%)	15100-043	Gibco BRL, USA
Sodium chloride (NaCl)	S5886	Sigama-Aldrich,USA
Sodium DL-lactate (60% syrup)	L4263	Sigama-Aldrich,USA
Sodium pyruvate	P3662	Sigama-Aldrich,USA
Weak iodine solution	-	ICN Bioedicals, USA
Xylazine hydrochloride (Ilium Xylazil-20)	L10600	Troy Laboratories, Australia

Appendix Table 1.3: List of labwares and disposables

Lab-wares and disposables	Manufacturer
Aluminium foil	Reynolds Consumer Products, USA
Autoclave disposal bag	Megalab supplies, Malaysia
Blades (Super Nacet)	Gillette, USA
Chromic catgut and other suture aterials	Aesculap®, Germany
Culture dish	Nunc, Denark
Disposable glass Pasteur pipette	Hirschmann®, Laborgerete, Germany
Disposable hand tissues	Megalab supplies, Malaysia
Falcon™ conical tube	Becton Dickinson, USA
Foley Catheters	Bardia®, India
Glassware (beaker, flask, easuring cylinder ect.)	Pyrex® , Japan
Lens cleansing tissue (Kiswipe® EX-L)	Kiberly-Clark, USA
Micropipette tips without filter	Axygen Scientific,USA
Needle	Terumo Corporation, Japan
Parafilm	Pechiney Plastic Packaging, USA
Schott bottle	Duran, Germany
Sterile glove	Ansell International, Malaysia
Syringe	Terumo Corporation, Japan

APPENDIX 2: TABLE OF STATISTICS ON ANALYSIS OF VARIANCE (ANOVA)

Appendix Table 2.1: Summary of analysis of variance on the overall number (mean ± SEM) of CL, oocytes and embryos obtained during surgery from donor does treated with PMSG and FSH-V

Descriptives

		N	Mean	Std. Deviation	Std. Error	95% Confidence Interval for Mean		Minimum	Maximum
						Lower Bound	Upper Bound		
No. of CL	PMSG	40	7.7750	7.46097	1.17968	5.3889	10.1611	.00	27.00
	FSH-V	16	11.5000	5.53775	1.38444	8.5491	14.4509	4.00	22.00
	Total	56	8.8393	7.12174	.95168	6.9321	10.7465	.00	27.00
No.of oocytes plus embryos obtained	PMSG	40	2.6500	4.02906	.63705	1.3614	3.9386	.00	13.00
	FSH-V	16	5.0625	3.60497	.90124	3.1415	6.9835	.00	13.00
	Total	56	3.3393	4.03294	.53892	2.2593	4.4193	.00	13.00
No. of embryos obtained	PMSG	40	.6250	1.47956	.23394	.1518	1.0982	.00	6.00
	FSH-V	16	4.3750	3.38378	.84595	2.5719	6.1781	.00	11.00
	Total	56	1.6964	2.75628	.36832	.9583	2.4346	.00	11.00

ANOVA

		Sum of Squares	df	Mean Square	F	Sig.
No. of CL	Between Groups	158.579	1	158.579	3.255	.077
	Within Groups	2630.975	54	48.722		
	Total	2789.554	55			
No.of oocytes plus embryos obtained	Between Groups	66.516	1	66.516	4.338	.042
	Within Groups	828.038	54	15.334		
	Total	894.554	55			
No. of embryos obtained	Between Groups	160.714	1	160.714	33.752	.000
	Within Groups	257.125	54	4.762		
	Total	417.839	55			

Appendix Table 2.2: Summary of analysis of variance on the number (mean±SEM) of CL observed at different days prior surgery, during surgery and the number of oocytes plus embryos retrieved from donor does treated with PMSG and FSH-V

Descriptives

		N	Mean	Std. Deviation	Std. Error	95% Confidence Interval for Mean		Minimum	Maximum
						Lower Bound	Upper Bound		
Two Days CL	PMSG	15	1.4000	1.59463	.41173	.5169	2.2831	.00	4.00
	FSH-V	15	3.4000	1.29835	.33523	2.6810	4.1190	2.00	6.00
	Total	30	2.4000	1.75381	.32020	1.7451	3.0549	.00	6.00
Four Days CL	PMSG	15	2.1333	1.72654	.44579	1.1772	3.0895	.00	5.00
	FSH-V	15	4.0000	1.41421	.36515	3.2168	4.7832	2.00	7.00
	Total	30	3.0667	1.81817	.33195	2.3878	3.7456	.00	7.00
Surgery Day CL	PMSG	15	6.4667	6.51226	1.68146	2.8603	10.0730	.00	17.00
	FSH-V	15	11.9333	5.79984	1.49751	8.7215	15.1452	4.00	22.00
	Total	30	9.2000	6.66644	1.21712	6.7107	11.6893	.00	22.00
No.ofo oocytes plus Embryos obtained	PMSG	15	3.7333	4.47958	1.15662	1.2526	6.2140	.00	12.00
	FSH-V	15	5.4000	3.45998	.89336	3.4839	7.3161	.00	13.00
	Total	30	4.5667	4.02307	.73451	3.0644	6.0689	.00	13.00
No of Embryos obtained	PMSG	15	.7333	1.57963	.40786	-.1414	1.6081	.00	5.00
	FSH-V	15	4.6667	3.28778	.84890	2.8460	6.4874	.00	11.00
	Total	30	2.7000	3.22864	.58947	1.4944	3.9056	.00	11.00

ANOVA

		Sum of Squares	df	Mean Square	F	Sig.
Two Days CL	Between Groups	30.000	1	30.000	14.189	.001
	Within Groups	59.200	28	2.114		
	Total	89.200	29			
Four Days CL	Between Groups	26.133	1	26.133	10.493	.003
	Within Groups	69.733	28	2.490		
	Total	95.867	29			
Surgery Day CL	Between Groups	224.133	1	224.133	5.895	.022
	Within Groups	1064.667	28	38.024		
	Total	1288.800	29			
No. of oocytes plus Embryos obtained	Between Groups	20.833	1	20.833	1.301	.264
	Within Groups	448.533	28	16.019		
	Total	469.367	29			
No of Embryos obtained	Between Groups	116.033	1	116.033	17.442	.000
	Within Groups	186.267	28	6.652		
	Total	302.300	29			

Appendix Table 2.3: Summary of analysis of variance on the number (mean ± SEM) of oocytes plus embryos retrieved from donor does treated with FSH-V in two different localities

Descriptives

		N	Mean	Std. Deviation	Std. Error	95% Confidence Interval for Mean		Minimum	Maximum
						Lower Bound	Upper Bound		
No. of oocytes plus Embryos obtained	FSH-V Kuala Lumpur	16	5.0625	3.60497	.90124	3.1415	6.9835	.00	13.00
	FSH-V Kunming	45	9.4667	4.26721	.63612	8.1847	10.7487	.00	17.00
	Total	61	8.3115	4.51863	.57855	7.1542	9.4688	.00	17.00
No. of Embryos obtained	FSH-V Kuala Lumpur	16	4.3750	3.38378	.84595	2.5719	6.1781	.00	11.00
	FSH-V Kunming	45	8.1333	4.64465	.69238	6.7379	9.5287	.00	17.00
	Total	61	7.1475	4.63262	.59315	5.9611	8.3340	.00	17.00

ANOVA

		Sum of Squares	df	Mean Square	F	Sig.
No. of oocytes plus Embryos obtained	Between Groups	228.944	1	228.944	13.560	.001
	Within Groups	996.137	59	16.884		
	Total	1225.082	60			
No. of Embryos obtained	Between Groups	166.722	1	166.722	8.775	.004
	Within Groups	1120.950	59	18.999		
	Total	1287.672	60			

APPENDIX 3: PERMISSION LETTER

Appendix letter: Permission to use data from YunNan Agriculture University, China for M.Sc. Research (Xiao Zhi Chao, SGR090154)

21 June 2011

Prof. Dr. Ramli Abdullah
Institute of Biological Sciences
Faculty of Science
University of Malaya
50603 Kuala Lumpur
Malaysia

Dear Prof. Ramli:

Permission to Use Data from YunNan Agricultural University, China for M.Sc. Research (Xiao Zhi Chao, SGR 090154)

I would like to refer to our previous discussion regarding to the above.

I am very happy to give the permission to Xiao Zhi Chao (SGR 090154) who is a M.Sc. candidate from Faculty of Science, University of Malaya to use the data from Yunnan Agricultural University, China as a part of his research data for his thesis requirement. This is in accordance with our agreement on "Memorandum on Academic Exchange between University of Malaya and Yunnan Agricultural University, 2010-2015". The details of the data are as mentioned in the attachment.

I would like to wish him all the best in his studies and positive research outcome from this collaboration effort.

Thank you.

Prof. Ge Chang Rong
Vice Chancellor
Yunnan Agricultural University
China

APPENDIX 4: LIST OF PUBLICATIONS AND PRESENTATIONS

PRODUCTION OF GOAT OFFSPRING BY TRANSFERRING *IN VIVO* DERIVED EMBRYOS THROUGH EMBRYO TRANSFER

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1.0 INTRODUCTION

The objective of this study was to develop a suitable protocol for the collection and transfer of goat embryos as well as to produce goat kids through embryo transfer (ET). Goat plays a significant role in poverty alleviation, especially in the developing countries, where 95% of the world goat population is found. The overall goat production can be increased by the substitution of genetically superior animals for those of little genetic merit. At present, ET is a powerful tool for multiplication of superior animals.

2.0 OBJECTIVES

a) To develop a suitable protocol for the collection and transfer of goat embryos.

b) To examine the survivability of goat embryos after ET.

c) To produce goat kids through ET.

d) To evaluate the effects ET recipients on ET performance

3.0 MATERIALS AND METHODS

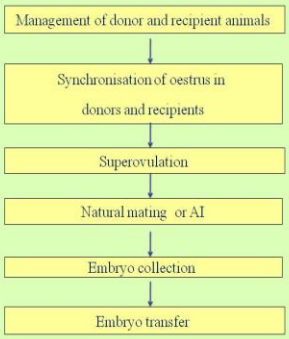
All does were undergoes synchronization of estrus and superovulation to obtain the embryos. For donor goats, the treatment was 14 days CIDR and upon CIDR withdrawal, 1200 IU PMSG was injected followed by checking estrus and natural mating. After day-7 CIDR withdrawal, uterine horn was flushed surgically to collect embryos. For recipient goats, similar CIDR treatment was given and after upon CIDR withdrawal, 200 IU PMSG was injected. On the same day, the collected embryos were transferred into the uterus of the recipient, in which it was confirmed by appearance of 1 to 2 corpus lutea on the ovary.

3.1 Experimental animals

a) Doe goat(Figure 1)

b) Buck goat(Figure 2)

3.2 Flow of experiment



3.3 Experimental animals and samples

Figure 1: (a) Boer does for experiment.
(b) Boer buck for experiment.

Figure 2:Hormone

Figure 3: Nature mating and artificial fertilization(AI).

Figure 4: Flushing embryos from the donor Doe

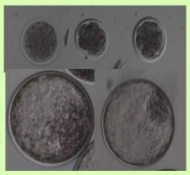


Figure 5: Different stage of embryos and oocyte

Figure 6: Transfer the fresh embryos in recipient doe uterine horn.

Figure 7: The kid and recipient

4.0 RESULTS

Table 1: Summary of embryos/oocytes obtained from donor does

Outputs	No. of Donor	Percentage
Embryos	6/41	14.6%
Unfertilised oocytes	11/41	26.8%
No. embryos/oocytes	24/41	58.53%

Table 2: Summary of result for goat synchronisation and superovulation

No. of oocytes/ embryos recovered	Stage.	No. of oocytes/embryos	Percentage of oocytes/embryos
	Unfertilised	75	77.32%
	2-cell	3	3.1%
	4-cell	1	1.1%
	8-cell	6	6.19%
	16-cell	3	3.09%
	Morula	6	6.32%
	Blastocyst	3	3.09%
Total		97	

Table 3: The rate of embryo pregnancy and kidding

Number of recipients	Number of embryos transferred	Pregnancy rate(pregnancy recipients)	Embryos survival (kidding number)
6	16/6	33.33% (2/6)	18.75% (3/16)

5.0 DISCUSSION

From the results obtained, the number of transferable embryos (blastocyst is the remarkable as transferable embryos) is much lower if compared to the number of unfertilised oocytes collected from the donor does. According to the findings, multiple ovulation embryo transfer (MOET) technologies is one of the reproductive methods to collect more embryos in one time which can produce approximately 6-8 of transferable embryos per goat[1] and the rate of pregnancy after transferred is between 40-80%[2] .

There were a few factors influences the outcome of this study at present. At first place, using the different hormone to superovulate the goats will get different results. Recovery of embryos was significantly higher ($P < 0.001$) with FSH-P (4.72±4.33) than with PMSG (2.50±5.02) treatment[3] ; in the second place, the seasonal effect on superovulatory treatment. There is huge a difference of the superovulatory result for goat in breeding season or no breading season, a 100% response rate to oestrus synchronisation treatment was found in doelings (n = 15, 2 of which were repeats) treated early in the breeding season, and the superovulation regimen resulted in the surgical collection of an average of 15.1 viable embryos per doeling[4]. Malaysia is a tropical climate country, perennial temperature is keep in 22-34°C, therefore, for the goat, there is not traditional breeding season; in the third place, the effect of transportation temperature on pregnancy goat, researchers found when embryos were incubated at 20°C for transportation, embryo survival and pregnancy rates were significantly lower than at 36.5°C and 38.5°C[5] .

Futhermore, at present, there are still many unanswered queries pertaining to the effect on superovulation and rate of pregnancy.

6.0 CONCLUSIONS

We successfully obtained 22 embryos from 41 donor does after superovulation and mating. However, we only manage to transfer 16 embryos into 6 recipients. Out of the 16 transferred embryos, we obtained 3 kids through MOET method in Malaysia. It is hoped that with further refinement, this embryos transfer technique could be routinely used to conserve as well as commercially produced of goat in the future in Malaysia.

REFERENCES

1) Cognie Y, Ba G, Poulin N, Beckers JF. 2000. The ovulation rate obtained after a superovulatory treatment associating GnRH antagonist and pFSH is highly repeatable. Proc 16th Mth Asso Euro Trans Embryo(AETE), 130
2) Mani AU, Waston ED and McKelvey WAC. 1994. The effects of subnutrition before or after embryo transfer on pregnancy rate and bmbryo survival in does. Theriogenology, 41: 1673-1678
3) S.Mahmood,G.L.Koul and J.C.Biswas.1990. Comparative efficacy of FSH-P and PMSG on superovulation in Pashmina goats.Theripgenology Volume 35, Issue 6, June 1991, Pages 1191-1196
4) Senn BJ and Richardson ME. 1992 Sasonal effect of caprine response to synchronozation of estrus and superovulatiory treat. Theriogenology,37: 579-585
5) Fusheng Quan, Zhiping Zhang, Zhixin An, Song Hua, Min Wan, Xiao'e Zhao, Yong Zhang. 2010. Effent of transpoting donor or recipient does and their embryos on the out come of fresh embryo transfer in Boer goats. Small Ruminant Research 88(2010) 1-5

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