CHAPTER II
LITERATURE REVIEW

2.1 Reproductive physiology of Thai-native goat

Small ruminant (sheep and goat) production is becoming such an important livestock species in Thailand. The average numbers of offspring from natural mating in Thai native does were reported from Saithanoo et al. (2001), with the ratio of singleton: twin: triplet was 17.6: 65.9: 16.5, respectively. Multi-pregnancy rate in Thai native does were 83.5%. Additionally, the litter size and multiple pregnancy rate will be increase in the next parturition. However, incidence of reduce in fetal weight and high mortality rate related with placental weight in multiple pregnancy model of compromised pregnancy in sheep. Because of increased number of fetuses was associated with reduced uterine blood flow per fetus, decreased placentome numbers and total placentome weight per fetus, decreased fetal weight, and increased neonatal mortality (Vonnahme et al., 2008).

Thai-native does can be used for breeding when they reach 8-10 months of age (Pralomkarn et al., 1996) and reach puberty when their body weight is more than 17.8 kg (Lertchunhakiat, 2012). Goats in Thailand are not seasonal breeders unlike those in the temperate zone and showing estrus with ovulation in all months of the year. Indigenous does first come into estrus as early as 3-4 months of age. The estrous cycle in general ranges from 18-22 days (Pralomkarn et al., 1996). Ovarian activity was observed in Thai-native goats run either alone, continuously with males, or intermittently with males at 56 day intervals between June 1985 and July 1986. Overall, the incidence of ovulation was highest in October and December (78.8%) and lowest in June (54.3%), with some does ovulating at every observation. The presence of males, either continually or intermittently, significantly increased the proportion of does ovulating throughout the year. The ovulation rate varied throughout the year, with the highest rate occuring in October 1985 (1.9) and the lowest rate in May and July 1986 (1.3) (Suttiyotin et al., 1991).

Pregnancy status was determined in two groups of Thai-native goats, mated in either October (n = 116) or March (n = 37), by assay of the progesterone level in four

plasma samples taken at 7 day intervals after the completion of mating. The progesterone (P4) level in each sample was determined using facilities in a local hospital, and a commercial assay kit with human serum-based standards was used. The distribution of log10 P4 yielded a discrimnatory value of 2 ng/ml; any value below this level was assumed to indicate a follicular phase. Pregnancy diagnoses based on this criterion were 96.2% accurate. Diagnoses based on returns to service were not accurate, as 36.5% of pregnant does were recorded as returning. Real-time ultrasonic imaging of the March mated group was 100% accurate for pregnancies, but detection of twins was poor. The P4 technique described here is useful in field studies where mating dates are not known, and where there is no access to an animal assay laboratory (Restall et al., 1990).

2.2 Follicular dynamic of goat

Follicular dynamics during the estrous cycle of the goat were first reported by Ginther and Kot (1994) using real-time ultrasonography. These authors frequently found four follicular waves in the estrous cycle and suggested that follicular dominance occurred in first and last waves. These results have been confirmed by other reports (Gonzalez de Bulnes et al., 1999), and are similar to the follicular dynamics observed in other ruminant species (Adams, 1999; Ireland et al., 2000; Mihm et al., 2002).

The ovulatory follicle was present at the time of the induced luteolysis in a significant proportion of the goats in the study of Gonzalez-Bulnes et al. (2005). Studies of follicular dynamics in the natural estrous cycle of goats (Ginther and Kot, 1994; Medan et al., 2003) show that there are no significant differences between the last two waves of the cycle or between these waves and the previous ones. However, these studies show a great variability in the number of follicular waves and it may be important to characterize them in relation to the number of waves present in the estrous cycle. The data of these studies were analyzed by combining the waves of each ovary, since both ovaries receive the same hypophyseal-pituitary signal, although the asymmetry of the follicular dynamics between right and left ovary has been recognized (Driancourt, 2001).

The mean interovulatory interval was 20.7±1.0 days (mean±SD). The interovulatory cycle of goats is characterised by a wavelike pattern of follicular development (Ginther and Kot, 1994). A follicular wave was defined by consecutive days of entry of follicles ≥6 mm into the wave, and the day of emergence was defined as the first day that the ≥6 mm follicles were 3 mm. In 15 of 20 (75%) interovulatory intervals, 1 wave emerged during each of day 2 to day 1 (wave 1); days 2 to 5 (wave 2); days 6 to 9 (wave 3); and day 10 to 15 (wave 4). Ovulation occurred during wave 4. The mean days of emergence of waves 1 to 4 were day −1, 4, 8 and 13, respectively. However, in 5 of these 15 interovulatory intervals, 50% of the apparent waves merged or were continuous so that a distinction could not be made between 2 waves.

The largest follicle grew to a larger ($P<0.05$) maximum diameter for waves 1 (8.7±0.3 mm) and 4 (9.7±0.3 mm) than for waves 2 (7.2±0.2 mm) and 3 (7.3±0.2 mm). The following observations suggested that the phenomenon of follicular dominance was more common during waves 1 and 4 than during waves 2 and 3: 1) the interwave intervals (days) were longer ($P<0.05$) for waves 1 (3.4±0.2) and 4 (4.3±0.6) than for waves 2 and 3 (2.5±0.2 for each wave) and 2) the correlation between maximum diameter of largest follicle and the subsequent interwave interval was significant for waves 1 and 4 but not for waves 2 and 3. The 5 remaining interovulatory intervals were irregular and involved more than 4 waves, including 2 interovulatory intervals with prolonged follicular phases (14 and 21) and failures of ovulation (Ginther and Kot, 1994).

The results of daily ultrasonographic studies indicate that the interovulatory cycle of goat is characterized by a wavelike pattern of follicular development (Ginther and Kot, 1994; de Castro et al., 1999) as was reported for other ruminant species (Sirois and Fortune, 1988; Ginther et al., 1995). A follicle wave 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; Schwarz and Wierzchos, 2000; Menchaca et al., 2002). The emergence of waves 1, 2, 3 and 4 (the

ovulatory wave) occurs on day 0, 5–6, 10–11 and around day 15 post-ovulation, respectively (Figure 2.1). In goats that developed three follicular waves, wave 2 emerges 1–2 days later and the ovulatory wave emerges 1–2 days earlier (Figure 2.1).

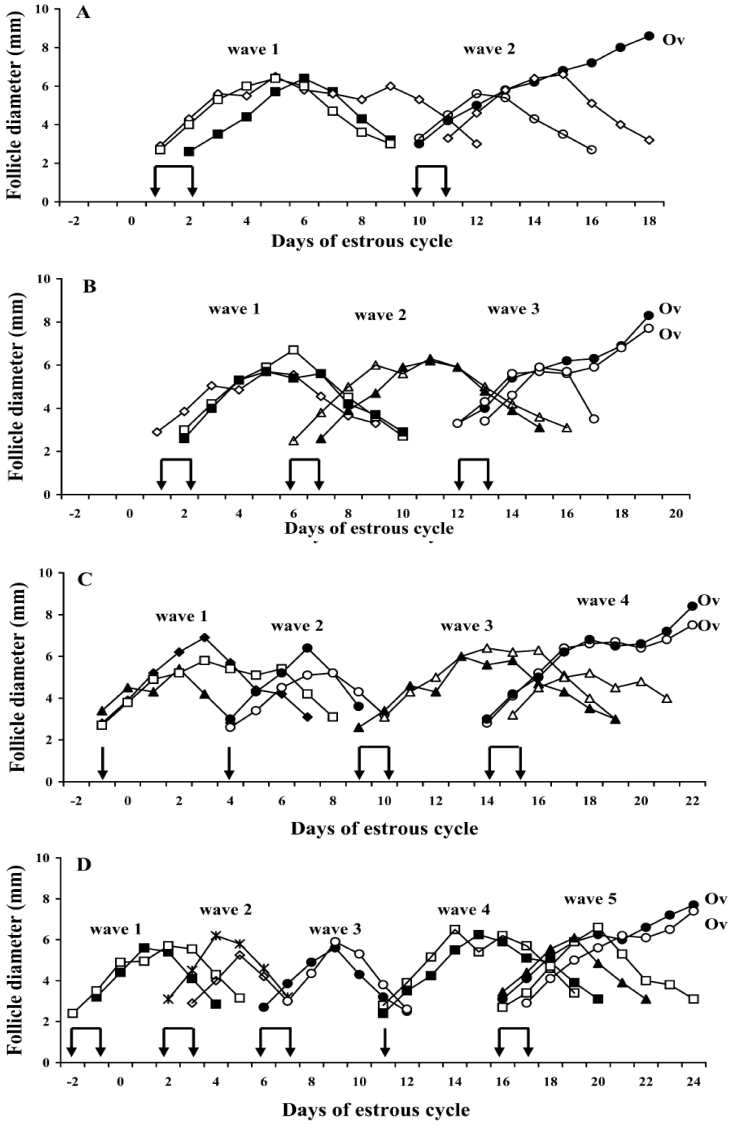

Figure 2.1 Representation patterns of growth and regression of individual follicles during the estrous cycle in goats with two (A), three (B), four (C), and five (D) waves of follicular development. Arrows indicate the emergence of follicular waves, and different symbols indicate different follicle in each follicular wave (Ov = ovulation)

Source: Medan et al. (2003)

The occurrence of reduced, or lack, of follicular dominance in some waves in the middle of the estrous cycle of polyovular species (sheep and goats) has been reported by some authors (Schrick et al., 1993; Orita et al., 2000), but there is no agreement on this point (Bartlewski et al., 1999; Gibbons et al., 1999; Evans et al., 2000). In cows, the pattern of hormonal and follicular dynamic of waves with co-dominant follicles or one dominant follicle has some differences (Kulick et al., 2001) to small ruminants.

Rubianes and Menchaca (2003) suggested that the morphological and functional characteristics of ovarian structures have provided an understanding of the relationships between P4, estradiol (E2), Follicle stimulating hormone (FSH) or inhibin to goat ovarian dynamics. Moreover, Rubianes and Menchaca (2003) suggested that when ovulation is induced by luteolysis the ovulatory follicle could be dominant follicle of an existing wave in either the growing or static phase. The ovulatory follicle was present at the time of the induced luteolysis in a significant proportion of the goats in the study of Gonzalez-Bulnes et al. (2005).

2.3 Estrous cycle and endocrine regulation of the goat

The estrous cycle consists of all morphological and physiological changes in the ovaries and genital tract leading to estrous expression (phase of receptivity towards males) and ovulation and the preparation of the genital tract for copulation, fertilization and embryo implantation (Fatet et al., 2011). The complete estrous cycle in goat is divided into 4 well marked phases, namely proestrus, estrus, metestrus and diestrus (Rahman et al., 2008). The length of estrous cycle is defined by the interval between two successive expressions of estrus or two successive ovulations. While the average duration of the goat estrous cycle is of 21 days (Figure 2.2), its length is highly variable. A study with Alpine goats during the breeding season recorded 77% cycles of normal in duration (17–25 days), 14% were short cycles (8 days in average) and 9% were long cycles (39 days in average; Baril et al., 1993). During the estrous cycle, ovaries undergo a number of morphological (follicular recruitment and growth), biochemical (follicle maturation) and physiological (endocrine regulations) changes leading to the ovulation. These cyclical changes in the gonads are referred to as the ovarian cycle. Follicular growth evolves in a wave-like manner throughout the

cycle (Figure 2.2). A follicular wave is characterized by the sequence of three gonadotropin-dependent events in follicular growth: recruitment, selection and dominance (Driancourt, 2001).

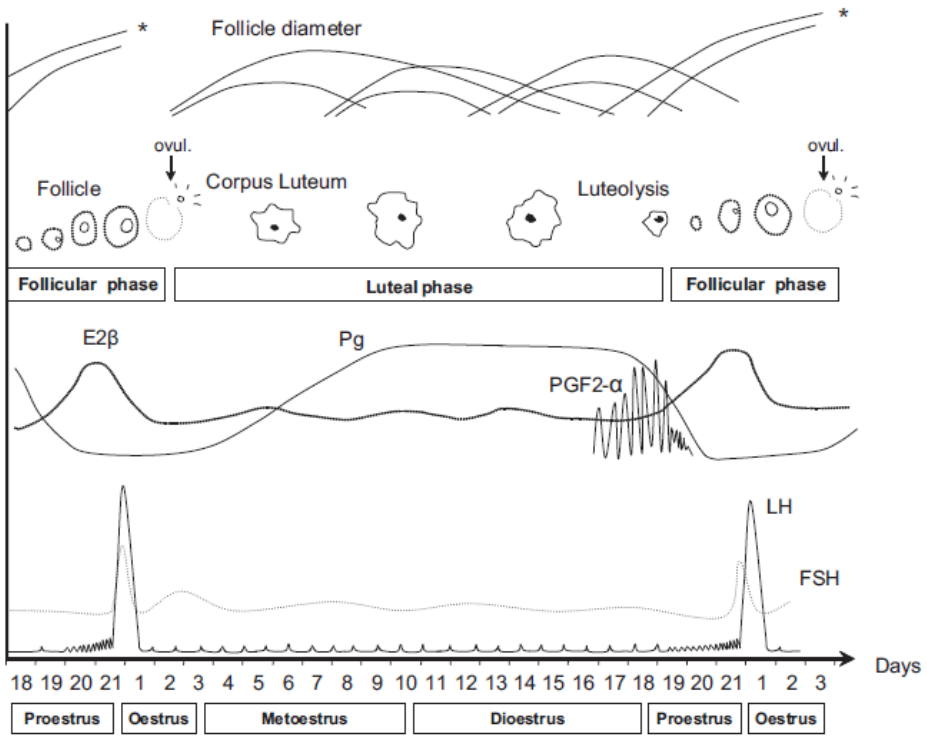

Figure 2.2 Schematic representation of the different physiological events occurring during estrous cycle in goat: pattern of follicle development, ovarian cycle and endocrine regulations

*Ovulatory follicle(s)

Source: Fatet et al. (2011)

The ovarian cycle is classically divided in two phases: the follicular phase and the luteal phase (Figure 2.2). The follicular phase corresponds to the wave of follicle development providing the ovulatory follicle and involves maturation of gonadotropin-dependant follicles until ovulation (terminal growth). During the follicular phase, FSH secreted by the pituitary gland stimulates follicular growth. A cohort of gonadotropin-dependant antral follicles of 2–3 mm of diameter is recruited and follicles enter their terminal growth. Only 2–3 of these follicles reach 4 mm

diameter and are selected to enter the dominance phase. Under the influence of luteinizing hormone (LH), they reach the pre-ovulatory stage (6–9 mm), while subordinate follicles degenerate (follicular atresia). The increase in peripheral concentrations of estradiol 17ß, secreted by bigger follicles, induces estrous behavior and acts as a positive retro control on the gonadotropic axis. The consequent increase in gonadotropin releasing hormone (GnRH) secretion induces the pre-ovulatory LH surge which induces ovulation 20–26 h later and subsequently luteinization of follicular cells. The beginning of the follicular phase, before overt estrous behavior is observed, is also referred to as the proestrus. The estrus phase includes events from overt estrous behavior to ovulation (Figure 2.2).

The luteal phase starts from the time of ovulation. About 5 days after the onset of estrus, cells of the ovulating follicle turn into luteal cells and form the corpus luteum (CL). They secrete progesterone causing its concentrations to increase and remain at a high level (>1 ng/ml) during 16 days. During this luteal phase, gonadotropin-dependant follicular growth continues in a wave-like manner but progesterone inhibits ovulation. At the end of the luteal phase, 16–18 days after estrus, prostaglandin $F_{2\alpha}$ ($PGF_{2\alpha}$) secreted by the non-gravid uterus induces the CL regression - called luteolysis and the decrease of P4 secretion. The decrease of plasma concentrations of P4 gradually removes the inhibition of gonadotropic hormones secretion and a new follicular phase then commences (Baril et al., 1993). The luteal phase is also called the proestrus period, which can be divided in metestrus, when peripheral concentrations of P4 begin to rise, and diestrus, when peripheral concentrations of P4 are high up to the start of luteolysis.

The duration of estrous behavior is about 36 h but varies from 24 h to 48 h depending on age, individuals and breeds, season and the presence of a male. Angora goats and Mossi goats are known to have a short estrus lasting only 22 h and 20 h, respectively (Shelton, 1978). Creole goats exhibit 27 h of estrous behavior and French Alpine goats are reported to experience a 31 h estrus (Baril et al., 1993). In Boer goats, the mean duration of estrus period is about 37 h (Greyling, 2000) and it is of about 58 h in Matou goats in Central China (Moaeen-ud- Din et al., 2008).

2.4 Hormonal control of estrous cycle in goat

Reproduction of small ruminants can be controlled by several methods developed in the last decades. Some of these involve the administration of exogenous hormones that modify the physiological chain of events involved in the sexual cycle. Some others do not include hormones, but only 'natural methods', such as light control or exposure to a male. Estrus synchronization plays a major role in fixed time breeding, AI, LOPU for oocyte or embryo collection and embryo transfer (ET). The value of estrus synchronization is vital in does as the duration of both estrous cycle and estrus is variable and estrous detection cannot be accomplished safely without a buck (Jainudeen et al., 2000). This technique has been developed in the early 1960s and since then a number of synchronizing methods has been developed for goats. Estrus synchronization in livestock focuses on the manipulation of either the luteal or the follicular phase of the estrous cycle. In goat, the opportunity for control is greater during the luteal phase, which is of longer duration and more responsive to manipulation. Strategies can be employed to extend the luteal phase by supplying exogenous progesterone or to shorten this phase by prematurely regressing existing CL. Successful techniques must not only establish tight synchrony, but also provide an acceptable level of fertility upon AI or natural mating (Rahman et al., 2008; Wildeus, 2000). Administration of hormones, such as progesterone or its analogues (progestagens) and prostaglandins, will modify the luteal phase of the cycle (Abecia et al., 2012).

2.4.1 Progesterone and analogues (progestagen)

The use of progestagens to induce estrus has allowed the increased use of artificial insemination in these species since the 1970s (Robinson et al., 1970), which affords an enormous advantage considering the difficulty of detecting estrus in these animal species. Methods which utilize progesterone or its analogues are based in their effects on the luteal phase of the cycle, simulating the action of natural progesterone produced in the corpus luteum after ovulation, which is responsible for controlling LH secretion from the pituitary. Thus, control of the life of the corpus luteum or manipulation of circulating progesterone concentrations allows for

regulation of estrus and ovulation (Hansel and Convey, 1983). In some subsequent investigations, pregnant mare serum gonadotrophin (PMSG) and human chorionic gonadotrophin (hCG) were administered in addition to progesterone treatment (Braden et al., 1960). The most common protocol for estrus synchronization in sheep/goats is based on progestagen/progesterone treatment (Abecia et al., 2011). The progesterone or progestagen treatment can be delivered through an intravaginal sponge, a Controlled internal drug release (CIDR) device or a norgestomet ear implant and another synthetic progestagen (melengestrol acetate, MGA) feed supplement) (Evans and Maxwell, 1987; Ritar et al., 1989; Freitas et al., 1997; Wildeus, 2000).

Intravaginal sponges have been the traditional treatment of choice for estrus synchronization in small ruminants, during the breeding and anestrus seasons. They are impregnated with progestagens that are effective at lower dose levels than natural progesterone. Two types of sponges are currently commercially available, based on either flurogestone acetate (FGA), marketed as chronogest (Intervet, Angers, France), or medroxyprogesterone acetate (MAP) (Rahman et al., 2008). Although sponges are widely used either in conjunction with PMSG, FSH or prostaglandin to more tightly synchronize and/or induce a superovulatory response, but sponges are not preferred as these frequently cause discomfort and may adhere to the vaginal wall causing problems with removal (Holtz, 2005).

An alternative means of supplying continuous, exogenous P4 has been the CIDR devices contains 0.30 g progesterone. CIDR are intravaginal devices constructed of a progesterone impregnated medical silicone elastomer moded over a nylon core has also been used usually impregnated with natural P4 (Wheaton et al., 1993). The device was designed in New Zealand in the late 1980s and is currently marketed in a number of countries. The devices are used for the reproductive manipulation of cattle (CIDR-B), sheep (CIDR-S) and goats (CIDR-G). Its use induces synchronized ovulation and estrous response (Wheaton et al., 1993; Whitley and Jackson, 2004). Plasma P4 concentrations increase rapidly after insertion of the device, reach highest concentrations 3 days after insertion and then gradually decrease similar to those of the natural estrous cycle (Wheaton et al., 1993).

The norgestomet ear implant supplied with the Syncro-mate-B (SMB; Rhone-Merieux, Athens, GA) developed for cattle. The cattle implant contains 6 mg of the synthetic progestagen norgestomet but is commonly used as one-half or one-third of the original implant when used in sheep and goats (Mellado and Valdez, 1997). Implantation periods for both species usually extend from 9 to 14 days and often are combined with PMSG and (or) $PGF_{2\alpha}$ co-treatments at or 2 days before the end of the implantation period.

MGA feed supplement, another synthetic progestagen, has also been used to induce estrus in sheep and goats (Abecia et al., 2012). This product is an orally active, developed and used for the suppression of estrus in feedlot heifers, but it has also been used for the induction of a fertile estrus in seasonally anovular ewes. The use of this product requires the feeding of supplement containing MGA (Pharmacia & Upjohn, Kalamazoo, MI) once or twice dairy for duration of 8 to 14 days (Wildeus, 2000). Moreover, stair-step feeding regimen affects fertility improvement in Thai-native goat (Nutthakornkul et al., 2010).

Currently the CIDR and subcutaneous implants are preferable than sponges because these are easy to use (Holtz, 2005) and CIDR does not absorb or obstruct drainage of vaginal secretions, resulting in less foul-smelling discharge upon removal (Motlomelo et al., 2002; Romano, 2004). However, costs of CIDR are relatively greater than use of sponge and may hinder widespread use of the CIDR. As a consequence, the development or refinement of efficient techniques that brings about cost reduction could be deemed appropriate in the case of estrus synchronization (Souza et al., 2011). Efficiency of synthetic progesterone/ progestagen products on estrus synchronization are summarized in Table 2.1

Table 2.1 Estrus and conception rate in goats synchronized with synthetic progesterone products

Types	Duration (day)	Associated treatment	Estrus (%)	Pregnancy rate (%)	Reference
FGA	13	5 mg $PGF_{2\alpha}$ at removal	100	63	Romano (2004)
	16	300 IU at removal	96.7	60	Motlomelo et al. (2002)
MAP	13	5 mg $PGF_{2\alpha}$ at removal	100	65	Romano (2004)
	16	300 IU at removal	93.1	51.7	Motlomelo et al. (2002)
CIDR	13	5 mg $PGF_{2\alpha}$ at removal	100	63	Romano (2004)
	5	10 mg lutalyse and 300 IU eCG at device insertion and removal	100	75.3	Vilariño et al. (2011)
	9	100 IU eCG and 0.05 mg cloprostenol at removal	100	95	Oliveira et al. (2001)
MGA	14	None	74	27.7	Quispe et al. (1994)
Syncro-mate-B	9	250 IU PMSG 48 h before removal	93	64	East and Rowe (1989)
	9	2.5 mg estradiol at implantation	100	80	Oliveira et al. (2001)

2.4.2 Re-use controlled internal drug release (CIDR) devices

The same CIDR devices, which contain P4, may be used for more than one treatment (Ungerfeld, 2009). In cattle, CIDR-B (1.9 g of P4) still contained P4 after its use (Van Cleeff et al., 1992), with the amount dependent on the duration of insertion. Although not recommended by the manufacturer, device re-use is a common practice in dairy herds. Re-use of P4 intravaginal devices have been reported in cows (Colazo et al., 2004), ewes (Ungerfeld, 2009) and goats (Oliveira et al., 2001) usually without decreasing fertility rate. Goats that are expressing estrous cycles in typical patterns receiving new or re-use CIDR devices showed similar estrous response and pregnancy rates with second (Oliveira et al., 2001) or third uses (Nogueira et al., 2011). Vilaiño et al. (2011) reported that goats received a short-term protocol for 5 days using CIDR of first, second and third use; the protocols induced estrus behavior and ovulation in 100% of goats in all groups and the onset of estrus, time of ovulation, and ovulation rate were similar for devices of first, second and third use. However, pregnancy rate was significantly lower for devices of third use compared with devices of first use. Similarly, Souza et al. (2011) cited that in anestrous goats it was possible to re-use CIDR devices to synchronize and induce estrus. Thus, the re-use of intravaginal devices as a cost-saver, is a possibility to be explored (Vilaiño et al., 2010).

2.5 Artificial insemination (AI) in goat

AI has changed the small ruminant industry and has allowed increased genetic improvement, better control of reproduction and sexually transmitted diseases, dissemination of valuable genetics and preservation of the genetics of endangered breeds (Cseh et al., 2012). AI was the first great biotechnology applied to improve reproduction and genetics of farm animals. It has had an enormous impact worldwide in many species. The acceptance of AI technology worldwide provided the impetus for developing other technologies, such as cryopreservation and sexing of sperm, estrous cycle regulation, and embryo harvesting, freezing, culture and transfer, and cloning (Foote, 2002).

The success of an AI program depends on many factors: whether fresh or frozen semen is used, number of times insemination during estrus, time and method of

insemination, and location (vagina, cervix, trancervical or uterus) of insemination, quality and quantity of semen inseminated (number of live sperm cells), semen (fresh or frozen) handling for AI, and management of the animals to be inseminated (Ax et al., 2000).

2.5.1 History of AI in goat

Leeuwenhoek (1678) and his assistant, Hamm, were the first persons to see sperm, which they called "animalcules". Leeuwenhoek did not have an advanced formal education, so he did not study Latin, the scientific language of the day. However, he was a clever, capable individual who ground lenses so precisely (one still exists today with 270 magnifications) that sperm were visible. His published paper (Leeuwenhoek, 1678) amazed, and perhaps amused, the reigning king of England, who regularly read papers submitted to the Royal Society, where Leeuwenhoek's paper was published. Another century passed before the first successful insemination was performed by Spallanzani (1784) in a dog, which whelped three pups 62 days later. Spallanzani originally trained to be a priest, but he had a great interest in natural history and pursued the latter.

The early development of AI in sheep and goat on a major scale began in Russia (Maule, 1962) where the collective farms provided an ideal arrangement for establishing AI programs. Then on, AI spread to central Europe and was widely applied commercially in France and Brazil (Maule, 1962). China also has extensive sheep AI programs (Foote, 2002). The techniques for semen collection and artificial insemination in sheep and goats have been described in detail (Evans and Maxwell, 1987).

2.5.2 The anatomy of the cervix

The use of frozen-thawed semen in conventional insemination yields poor fertility in sheep. This is due to different factors but one of the most important restrictive reasons is the anatomical structure of the cervix. The ovine cervix is a long, fibrous tubular organ composed predominantly of connective tissue with an outer serosal layer and inner luminal epithelium (Kershaw et al., 2005) and fibrous tube whose lumen is obstructed by prominences and depressions in the mucosal

membrane which from annular folds or rings in members of 3-7 (Kershaw et al., 2005; Naqvi et al., 2005). These folds narrow the cervix lumen and prevent the catheters from reaching the uterus (Bunch and Ellsworth, 1981; Halbert et al., 1990). Furthermore, the second fold is generally misaligned in relation to the others (Fukui and Roberts, 1978; Naqvi et al., 2005), and may block the progression of standard AI catheter to within the cervix. This does not happen in other similar species like goat in which the cervical lumen has more folds (Bunch and Ellsworth, 1981; Halbert et al., 1990; Kaabi et al., 2006). Thus, AI in goats can intrauterine insemination via the cervix is much easier than in ewes.

Appearances of the vaginal protrusions in the Angora goat were star (Figure 2.3/A), duckbill (Figure 2 .3/B), crescent (Figure 2.3/C), spiral (Figure 2.3/D), cluster (Figure 2.3/E) and bump-shaped (Figure 2.3/F). In the specimens examined, the star, duckbill, crescent, spiral, cluster and bump-shaped vaginal protrusions were 22, 6, 2, 6, 4, and 20 in number, respectively.

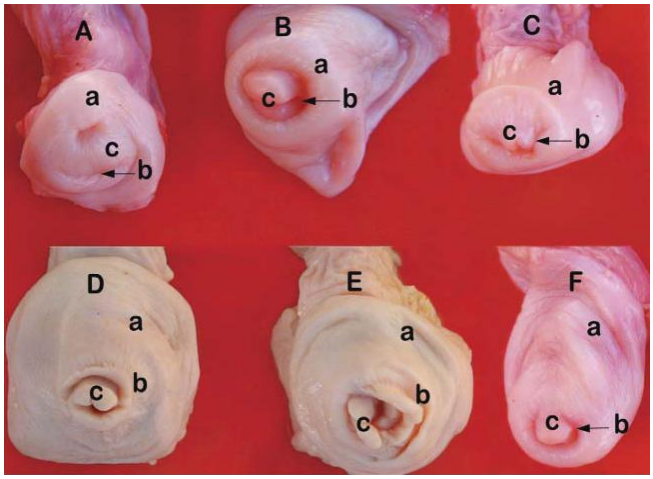

Figure 2.3 Appearances of the vaginal protrusions in the Angora goat; A. star, B. duckbill, C. crescent, D. spiral, E. cluster, F. bump, a. vaginal cavity, b. vaginal fornix, c. vaginal protrusion, d. external cervical orifice, e. funnel-shaped cervical folds with the smallest opening pointing caudally, f. blind sac, g. accentric cervical canal, g'. eccentric portion of the cervical canal, h. internal cervical orifice, i. uterine cavity

Source: Dayan et al. (2010)

Although the authors Halbert et al. (1990) and Kaabi et al. (2006) stated that the numbers of the cervical folds varied from 5 to 6, Dayan et al. (2010) found the mean number of cervical folds in Angora goats were 4.3. Hence, it may be suggested that artificial insemination can be more easily performed than that of the ewe.

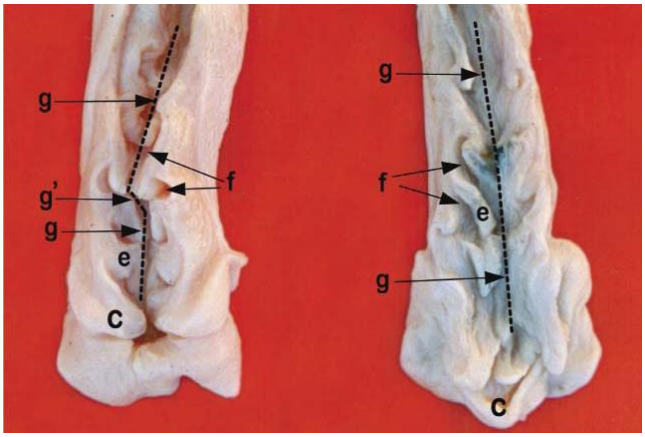

Figure 2.4 Longitudinal section of the cervical canal, letter as for Fig. 2.3
Source: Dayan et al. (2010)

The cervical canal of the Angora goat had a convexity in dorsal direction (Figure 2.5) because its cranial and caudal thirds were somewhat curved ventrally.

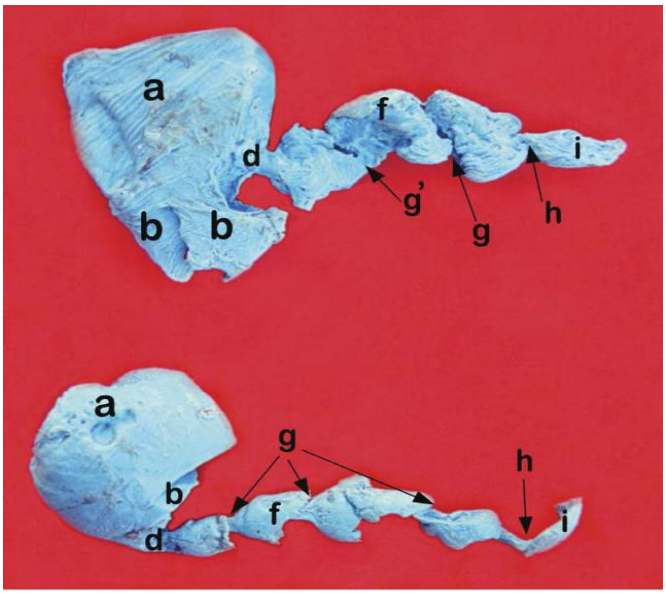

Figure 2.5 Latex casts of the cervical canal, letter as for Fig. 2.3
Source: Dayan et al. (2010)

2.5.3 Artificial insemination techniques

Technically, artificial insemination in goats is very similar to that of sheep, but to achieve an intrauterine insemination via the cervix is much easier in does than in ewes (Cseh et al., 2012). There generally are 3 AI techniques 1) vaginal insemination, 2) the cervical insemination and 3) the laparoscopic intrauterine insemination that have been used in small ruminants (Leethongdee, 2009) and newly developed fourth technique, a transcervical artificial insemination method based on experience acquired in the process of establishing a transcervical embryo collection technique for goats (Sohnrey and Holtz, 2000). The pregnancy rate and kidding rate following vaginal, cervical, laparoscopic, and transcervical artificial insemination are summarized in Table 2.2

Vaginal artificial insemination, this method involves depositing semen deep in vagina without any attempt to locate the cervix (Figure 2.6). Semen is deposited in the anterior vagina (Leethongdee, 2009). Vaginal insemination is effective in does with directly inseminated fresh semen, but gives poor results with extended (chilled semen) or frozen semen (Cseh et al., 2012).

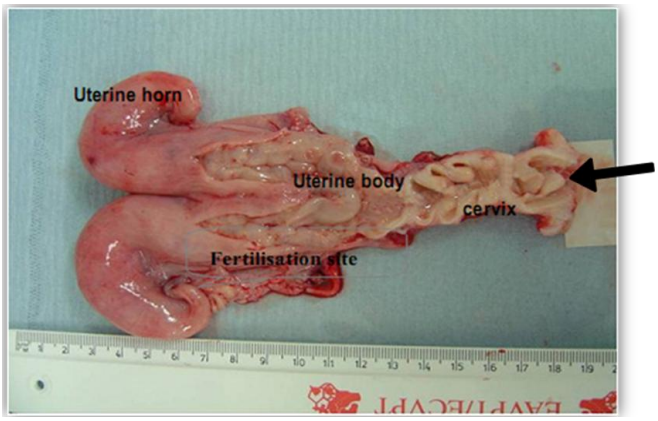

Figure 2.6 The vaginal artificial insemination, the semen is deposited in the anterior vagina

Source: Leethongdee (2009)

Cervical artificial insemination, the semen was deposited into the external os of the first cervical fold (Figure 2.7), using speculum fitted with an internal light source as described (Karagiannidis et al., 2001). Cervical insemination using fresh semen gives a higher pregnancy rate than using frozen-thawed semen (Donovan et al., 2004) and pregnancy rates after cervical insemination with frozen semen are higher in goats than ewes (Cseh et al., 2012).

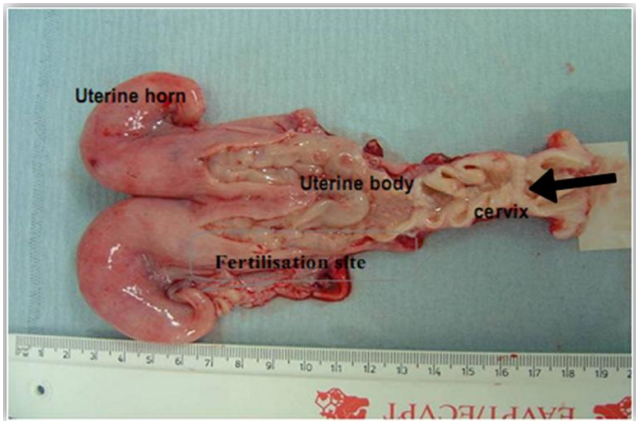

Figure 2.7 The cervical artificial insemination, the semen is deposited into the external os of the first cervical fold

Source: Leethongdee (2009)

Laparoscopic intrauterine insemination, the complex anatomy of the cervix limits the passage of an inseminating pipette into the cervical canal and causes difficulty with transport of spermatozoa through the cervix. The difficulty of cervical passage can be overcome by direct uterine insemination (Figure 2.8) using laparoscopy (Killeen and Moore, 1970). The number of spermatozoa is again 20×10^6 (minimum) per insemination dose (Cseh et al., 2012). Fertility and pregnancy rates are high with either fresh or frozen-thawed semen (Leethongdee, 2009). Laparoscopy has the disadvantage of requiring elaborate equipment and special skill (Sohnrey and Holtz, 2005), the equipment is expensive and easily damaged and it may become unacceptable on animal welfare grounds and legislation (Leethongdee, 2009).

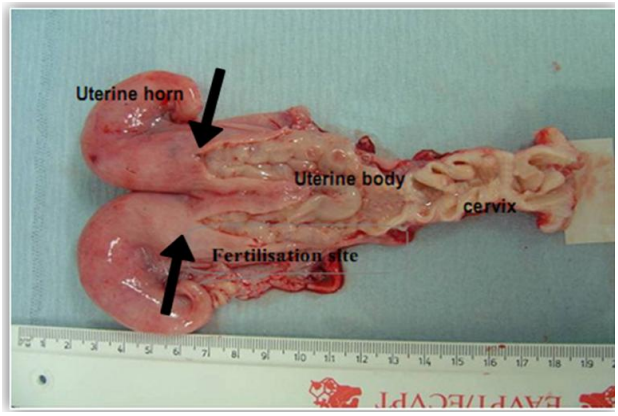

Figure 2.8 Laparoscopic intrauterine insemination, the semen is deposited into the uterine horn

Source: Leethongdee (2009)

Table 2.2 The pregnancy rate and kidding rate following the insemination technique compared among the artificial insemination (AI) techniques in goats

AI techniques	semen	Non-return rate (%)	Pregnancy rate (%)	Kidding rate (%)	Reference
Vaginal	fresh semen	74.3	-	-	Paulenz et al. (2005)
	frozen semen	64.3	-	-	Nordstoga et al. (2010)
Cervical	fresh semen	78.0	-	-	Paulenz et al. (2005)
	frozen semen	-	75.3	-	Vilariño et al. (2011)
		-	63.7	-	Menchaca and Rubianes (2007)
Transcervical	frozen semen	-	74	71	Sohnrey and Holtz (2005)
Laparoscopic	frozen semen	-	56	52	Sohnrey and Holtz (2005)

2.5.4 Transcervical artificial insemination (TCAI)

Transcervical artificial insemination (TCAI) is a method of insemination where semen is deposited deep in the cervix or even into the uterus via the cervix (Figure 2.9). This method involves depositing semen as deeply as possible in the cervix. The greater the depth of insemination, the higher the expected pregnancy and lambing rates (Salamon and Maxwell, 1995). There are varying degrees of damage to the cervical lining over the length of the cervix canal (Campbell et al., 1996). Alternatively, transcervical intrauterine insemination techniques (e.g., Guelph system of transcervical insemination) have been developed and improved to allow the semen to be deposited deeply into the uterine horns (Buckrell et al., 1994; Wulster-Radcliffe and Lewis, 2002; Wulster-Radcliffe et al., 2004; Sohnrey and Holtz, 2005). In ewes, the technique cannot be used with acceptable success in ewe-lambs. Cervical injury, abscesses, infections and resulting poor pregnancy rates are all associated with this technique. Pregnancy rates are generally lower than with laparoscopic insemination

(fresh semen: 40–80%; frozen semen: 30–70%) (Wulster-Radcliffe et al., 2004; Cseh et al., 2012).

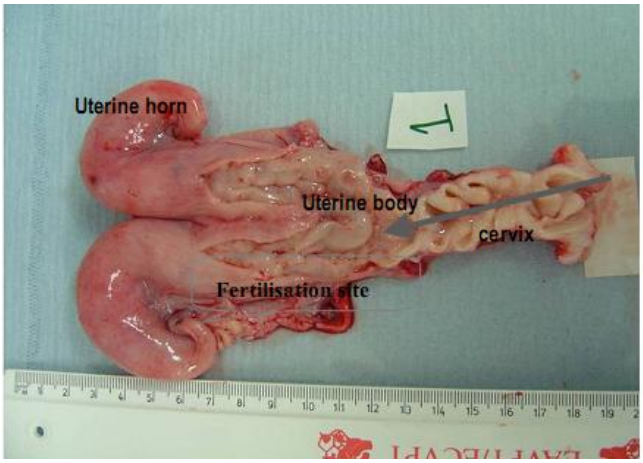

Figure 2.9 The transcervical artificial insemination, the semen is deposited into the cervix or uterus via the cervix

Source: Leethongdee (2009)

In goat, execution of transcervical insemination is very similar to that in ewes, but much simpler. The minimum required number of motile spermatozoa is 60×10^6 per insemination dose and the optimal time of insemination is between 49 and 65 h after removal of progesterone inserts (Cseh et al., 2012). Sohnrey and Holtz (2005) described a method by which semen can be deposited deep into the uterine horns through the transcervical route; the results are at least as good as that achieved by laparoscopy. It was, in all instances, possible to traverse the cervix and a 71% kidding rate was finally recorded and transcervically insemination may be considered a viable alternative to laparoscopic insemination.