

## **CHAPTER II**

### **LITERATURE REVIEW**

#### **2.1 Goat production in Thailand**

Thailand is the 50<sup>th</sup> largest country in the world with a total area of approximately 513,000 km<sup>2</sup> (198,000 mile<sup>2</sup>). It is the 21<sup>st</sup> most populous country with approximately 64 million inhabitants. The tropical climate in Thailand is dominated by monsoons and is characterized by high temperature and humidity. Although northern, northeastern, eastern and central Thailand have 3 seasons (summer, rainy and winter), southern Thailand has 2 seasons (summer and rainy). The national religion is Buddhism; however, approximately 10 percent of the population or 6.4 million inhabitants are Muslims, concentrated largely in the southern border provinces. Chickens account for the largest proportion of the country's livestock inventory in 2008 (84.92%), followed by ducks (8.19%), beef cattle (3.28%), swine (2.79%), buffalo (0.49%), dairy cattle (0.17%), goats (0.13%) and sheep (0.02%). The goat population in the country is relatively small (374,029); however, in the past 8 years, it has increased markedly at a rate of 22.74% per annum, especially in the central (42.12%) and Northeast (36.87%) parts of the country (Anothaisinthawee et al., 2010). Goats are predominantly raised by smallholders, particularly those in the Muslim community for religious and ritual slaughter. Farmers, classified as traditional or subsistence, commonly use low-input methods (such as graze crop residues or natural pasture) throughout the region. Goats in Thailand are predominantly used for meat production (90%), while milk production is a relatively small industry (10%). Goat raising is expanding following the demands of Malaysia as well as the government promotion strategies by the Department of Livestock Development (Anothaisinthawee et al., 2010). Thus, the research and development of technology for production, animal health, increasing product and accelerated extension of services to farmers are important. For the purpose of improving goat breeding, the DLD is now looking at genetic research to minimize production costs, optimize productivity and assist in accommodating consumer preference.

## 2.2 Reproductive physiology of Thai-native goat

Small ruminant (sheep and goat) production is becoming important to livestock production in Thailand. The average number of offspring from natural mating in Thai-native does was reported from Saithanoo et al. (2001), with the ratio of singleton: twin: triplet as 17.6: 65.9: 16.5. Multi-pregnancy rates in Thai native does is 83.5%. Additionally, the litter size and multiple pregnancy rate will increase at next parturition. However, incidence of reduction in fetal weight and high mortality rate related with placental weight in the multiple pregnancy model of compromised pregnancy in sheep. This is because an 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).

Ovarian activity was observed in native Thai goats running either alone, continuously with males, or intermittently with males at 56 days 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 occurring 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 native Thai 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 level (P4) 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 log<sub>10</sub> P4 yielded a discriminatory 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 progesterone 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).

### **2.3 Age at first estrus and induction of prepubertal period**

Female Thai-native goats reach puberty when their body weight is more than 13.25 kg. The onset of puberty is generally more related to body weight than to age (Nutthakornkul, 2011). Puberty can be defined as the first ovulation and/or first estrous behavior in females. Puberty in most goat breeds occurs generally at 5 to 10 months in does (Romano, 1998). Nevertheless, Breeding is delayed until the animal has attained 60.75% of its mature body weight (Sodiq et al., 2002).

The factors affecting the puberty and age at first estrus include genetic and environmental factors (Cedillo et al., 1977). For genetic factors, many reports have shown that there is an interbreed difference in the onset of sexual activity in does and bucks that are well fed. In does of the Shiba breed and in local female goats from the Caribbean Guadeloupe Island, puberty starts from 5.6 to 6.7 months of age, whereas the Saanen does attain puberty at 7.8 months of age (Delgadillo et al., 2007). Age at first mating of female goats ranges between a minimum of 7 months in the Kacang goats to a minimum of 2 years in Peranakan Etawah goats (Sodiq et al., 2002). In addition, there are environmental factors such as the level of nutrition, type of birth, season of birth, health, disease, and management system (Christopher, 2002). For example, Delgadillo et al. (2007) reported that there was an effect of the season on the date of first ovulation or puberty. In this case, does born in May have ovulatory activity which commences at an earlier age ( $201 \pm 3$  days) compared with does born in January ( $264 \pm 5$  days) and October ( $344 \pm 5$  days) groups ( $P < 0.001$ ).

The vasectomized buck was introduced to the doe herd for detecting heat and estrus behavior. There is evidence showing that the presence of the buck may modify the age of puberty in the goat and influence estrus behavior in does because “the buck effect” (Romano, 1998). Rosa and Bryant (2002) reported that the buck effect is one among various techniques available to manipulate reproduction during the anestrus season with the advantages of being inexpensive and free from hormone residue. Sexual performance of rams have efficacy to expressed sexual behavior and induction of estrus in ewes (Perkins and Fitzgerald, 1994) and Thai-native does (Nutthakornkul, 2011).

## 2.4 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-Bulnes et al., 1999; Padilla and Holtz, 2000), and are similar to the follicular dynamics observed in other ruminant species (Adams, 1999; Ireland et al., 2000; Mihm et al., 2002).

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 (Schrack 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.

Recent studies linking the morphological and functional characteristics of ovarian structures have provided an understanding of the relationships between progesterone (P4), estradiol (E2), follicle stimulating hormone (FSH) or inhibin to goat ovarian dynamics (Menchaca and Rubianes, 2002; Medan et al., 2003). Rubianes and Menchaca (2003) suggested that when ovulation is induced by luteolysis the ovulatory follicle could be the dominant follicle of an existing wave in either the growing or static phase.

The ovulatory follicle was presented 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  $20.7 \pm 1.0$  days (mean  $\pm$  SD). The interovulatory cycle of goats is characterised by a wavelike pattern of follicular development (Ginther and Kot, 1994).

The predominant follicular wave pattern in goats was 4 waves with ovulation from wave 4, and apparent follicular dominance was expressed during some follicular waves, especially during waves 1 and 4. Averaged over all interovulatory intervals, the number of 3 mm follicles on each day that later reached  $\geq 6$  mm followed a pattern of significant peaks on days 0 (ovulation), 4, 8 and 14, respectively.

A follicular wave was defined by consecutive days of entry of follicles  $\geq 6$  mm into the wave, and the day of emergence was defined as the first day that the  $\geq 6$  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 days 10 to 15 (wave 4). Ovulation occurred during wave 4. The mean days of emergence of waves 1 to 4 were days -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 ( $8.7 \pm 0.3$  mm) and 4 ( $9.7 \pm 0.3$  mm) than for waves 2 ( $7.2 \pm 0.2$  mm) and 3 ( $7.3 \pm 0.2$  mm). The following observations suggest 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 ( $3.4 \pm 0.2$ ) and 4 ( $4.3 \pm 0.6$ ) than for waves 2 and 3 ( $2.5 \pm 0.2$  for each wave) and 2) the correlation between the maximum diameter of the 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).

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## **2.5 Oocyte competence**

Oocyte competence can be defined as the ability of an oocyte to 1.) resume meiosis following gonadotropin stimulation, 2.) undergo cleavage divisions after fertilization, 3.) develop to the blastocyst stage, 4.) result in birth of live young and 5.) offspring with good health (Sirard et al., 2006).

The widespread use of a variety of assisted reproductive technologies has removed many of the constraints that previously restricted mammalian reproduction to the period between onset of puberty and reproductive senescence. In vitro embryo production systems now allow oocytes from very young animals to undergo fertilization and form embryos capable of development to normal offspring, albeit at somewhat reduced efficiencies compared to oocytes from adult females. They also can overcome infertility associated with advanced age of animals and women. This review examines oocyte developmental competence as the limiting factor in applications of assisted reproductive technologies for both juvenile and aged females. Age of oocyte donor is a significant factor influencing developmental competence of the oocyte. Age-related abnormalities of oocytes include a) meiotic incompetence or inability to complete meiotic maturation resulting in oocytes incapable of fertilization; b) errors in meiosis that can be compatible with fertilization but lead to genetic abnormalities that compromise embryo viability; and c) cytoplasmic deficiencies that are expressed at several stages of development before or after fertilization.

In general, oocytes from juvenile donors and the embryos derived there from appear less robust and may be less tolerant to suboptimal handling and in vitro culture conditions than are adult oocytes. Research to identify specific cytoplasmic deficiencies of juvenile oocytes may enable modifications of culture conditions to correct such

deficiencies and thus enhance developmental competence. Use of oocytes from aged donors for assisted reproduction can have a variety of applications such as extending the reproductive life of individual old females whose offspring still have high commercial value, and conservation of genetic resources such as rare breeds of livestock and endangered species.

In general, female fertility decreases with advancing age. Studies of women in oocyte donation programs have established reduced oocyte competence as the major cause of declining fertility with age, although inadequate endometrial function can also be a contributing factor. Most research has emphasized the importance of chromosomal abnormalities because of the well established increase in aneuploidy with increasing maternal age but little is known about the underlying cellular and molecular mechanisms.

Research aimed at identifying the specific developmental deficiencies of oocytes from juvenile donors and abnormalities of oocytes from aged females will assist in overcoming present bottlenecks that limit the efficiency of assisted reproduction technologies. Such research will also be the development of new oocyte-based technologies for overcoming infertility and possibly subverting chromosomal abnormalities in women approaching menopause (Armstrong, 2001).

## **2.6 Pregnancy establishment of domestic animals**

The establishment and maintenance of pregnancy results from signaling by the conceptus (embryo/fetus and associated extraembryonic membranes). The growth and development of the conceptus (embryo/fetus and associated extraembryonic membranes) in mammals unequivocally requires P4 and placental hormone actions on the uterus that regulate endometrial differentiation and function, pregnancy recognition signaling, uterine receptivity for blastocyst implantation, and conceptus-uterine interactions (Carson et al., 2000; Gray, 2001; Paria et al., 2000). Hormones from the conceptus act on the uterus in a paracrine manner to establish and maintain pregnancy.

Establishment of pregnancy involves maternal recognition of pregnancy and implantation. Maternal recognition of pregnancy is a phrase coined by Roger Short in 1969 and can be defined as the physiological process whereby the conceptus signals its presence to the maternal system and prolongs lifespan of the CL. In most mammals, P4

production by the CL is required for successful pregnancy. P4 acts on the uterus to stimulate and maintain uterine functions that are permissive to early embryonic development, implantation, placentation and successful fetal and placental development to term. Prolonged lifespan of the CL is a characteristic feature of mammalian pregnancy in species with a gestation period that exceeds the length of a normal estrous or menstrual cycle, such as domestic animals, laboratory rodents and humans.

Maintenance of pregnancy requires reciprocal interactions between the conceptus and endometrium. Available evidence supports the idea that hormones from the placenta act directly on the uterine endometrium to regulate cell differentiation and function. In domestic animals, the endometrial glands undergo a program of hyperplasia followed by hypertrophy that appears to be dependent on temporal and spatial actions of hormones from the placenta. Endometrial gland morphogenesis during pregnancy allows for the endometrium to increase output of secretory proteins that are transported to the fetus by specialized areas of the placenta termed areolae. Histotrophic nutrition from the endometrium is the first available nutrition for the developing conceptus and appears to be essential for conceptus survival and growth throughout pregnancy in domestic animals. This review summarizes current information on the biology of conceptus signals for establishment and maintenance of pregnancy, with particular emphasis on domestic animals (sheep and pig).

### **2.6.1 Pregnancy recognition signaling by interferon tau (IFN $\tau$ )**

Maternal recognition of pregnancy in ruminants (sheep, cattle, and goat) requires that the conceptus elongate from a sphere to a tube and then filamentous form to produce IFN $\tau$ , which is the pregnancy recognition signal that prevents development of the endometrial luteolytic mechanism (Spencer and Bazer, 2004). This antiluteolytic effect of IFN $\tau$  results in the maintenance of a functional CL and, hence, secretion of P4 that is essential to maintain a uterine environment that supports events critical to successful development of the conceptus to term.

Interferon tau (IFN $\tau$ ) produced by the embryonic trophoctoderm in the ruminant ungulates is known as a paracrine factor, which binds to the IFN receptor located at the uterine endometrium. Mechanisms to act antiluteolytic by 1) stabilization or up-regulation of progesterone receptors (PR) in the endometrium, 2) direct inhibition of endometrial estrogen receptors (ER), 3) direct inhibition of endometrial oxytocin receptors (OTR),

4) initiation of post receptor mechanisms, that prevent oxytocin induced release of PGF, and/or 5) inducing the endometrium to synthesize an inhibitor of enzymes necessary for the synthesis of PGF<sub>2α</sub> (Spencer and Bazer, 2004).

The maintenance of ovine corpus luteum during early pregnancy was shown to result from the production of a proteinaceous factor present in the pre-implantation conceptus. This factor, initially called trophoblastin, and later, ovine trophoblast protein 1 (oTP-1) is now known as ovine interferon-tau (oIFNτ). It was found to be the major secretory product of the ovine conceptus trophoblast cells between day 13 and day 16 of pregnancy, the time of maternal recognition of pregnancy in sheep. Similarly, a caprine interferon-tau (cIFNτ) was identified in goats, being secreted between days 16 to 21 of pregnancy. Since interferon-tau was not detected in peripheral blood of pregnant ewes, it is thought to act directly on the uterine endometrium (Spencer and Bazer, 1995).

During maternal recognition of pregnancy, the mononuclear cells of the conceptus trophoderm synthesize and secrete IFNτ between Days 10 and 21 to 25 with maximal production on Days 14 to 16 (Bazer, 1992). In terms of biological activity, a single Day 16 conceptus produces approximately  $1 \times 10^8$  antiviral units of IFNτ in culture over 24 h (Ashworth and Bazer, 1989). IFNτ appears to be the sole factor produced by the conceptus that prevents development of the endometrial luteolytic mechanism. IFNτ does not act to stabilize PR expression in the endometrial epithelium during pregnancy. Rather, IFNτ acts in a paracrine fashion on endometrial LE and sGE to suppress transcription of ERα and OTR genes, thereby abrogating development of the endometrial luteolytic mechanism. Indeed, the increases in ERα and OTR gene expression detected in luminal epithelium (LE) and germinal epithelium (GE) on Days 11 to 17 post-estrus in cyclic sheep do not occur in pregnant sheep (Spencer and Bazer, 1995) or in cyclic sheep infused with IFNτ (Spencer et al., 1995). By inhibiting increases in OTR expression, IFNτ prevents endometrial production of luteolytic pulses of PGF. However, IFNτ does not inhibit basal production of PGF, which is higher in pregnant than cyclic ewes, and the conceptus and IFNτ do not affect cyclo-oxygenase-II (COX-2) expression in the endometrial epithelia of early pregnancy sheep. Thus, the antiluteolytic actions of IFNτ are to prevent increases in epithelial ERα and OTR gene expression, which are estrogen responsive, by directly inhibiting transcription of the ERα gene and maintaining secretion of progesterone by the CL (Fleming et al., 2001).

IFN $\tau$  is a novel member of the Type I IFN family that acts differentially on the endometrial LE, GE and stroma to regulate expression of a number of IFN-stimulated genes (ISGs) that are hypothesized to play roles in endometrial differentiation and conceptus implantation (Spencer and Bazer, 2002). The actions of IFN $\tau$  to signal pregnancy recognition and induce or increase expression of ISGs, including ISG17 and 2', 5'-oligoadenylate synthetase (OAS) (Johnson et al., 2001), is dependent on the effects of progesterone. The Type I IFN receptor subunits, IFNAR1 and IFNAR2, are expressed in all endometrial cell types with highest expression in endometrial LE. However, the majority of ISGs are induced or increased in response to the conceptus or IFN $\tau$  only in the endometrial stroma and middle to deep GE of the ovine uterus (Spencer and Bazer, 2002). Interestingly, the induction of many ISGs is also observed in the porcine uterus during early pregnancy, and their expression is limited to the endometrial stroma. In the ovine uterus, the lack of ISG induction in the endometrial LE and sGE by IFN $\tau$  is apparently due to the expression of IFN regulatory factor two (IRF-2), a potent repressor of gene transcription that is constitutively expressed in the endometrial LE and sGE and increased during early pregnancy (Choi et al., 2001). In addition, IRF-2 appears to be involved in IFN $\tau$  inhibition of ER $\alpha$  gene transcription in the same endometrial epithelia (Fleming et al., 2001).

The finding that ISGs are induced in the underlying endometrial stroma led to the hypothesis that LE and perhaps GE produce an "interferonomedin" from the basolateral epithelial surface that acts as a paracrine amplifier of IFN $\tau$  responses in stroma. A more plausible explanation is that IFN $\tau$  produced by the conceptus may be transported across the LE cell layer or move passively into the underlying endometrial stroma. Guillomot et al. (1981; 1986) observed that horseradish peroxidase injected into the uterine lumen of pregnant sheep and cattle accumulated in the endometrial stroma beneath the basement membrane of the LE. This transport was mediated via both transepithelial endocytotic activity (vesicles) and passage through intercellular spaces between tight junctions. These phenomena were especially marked when systemic progesterone concentrations were high during late diestrus and when PR is absent from the endometrial LE. The precise nature of the crosstalk between progesterone and IFN $\tau$  remains undefined (Spencer and Bazer, 2004).

### 2.6.2 Implantation and establishment of pregnancy

Progesterone, the hormone of pregnancy, plays a pivotal and indisputable role in the establishment and maintenance of pregnancy in mammals. In all mammalian uteri, PR is expressed in the endometrial epithelia and stroma during the early luteal phase, allowing direct regulation of a number of genes by progesterone via activation of the PR. However, continuous exposure of the endometrium to progesterone down-regulates PR expression in the endometrial epithelium (Spencer and Bazer, 2004). Indeed, expression of PR protein is not detectable in endometrial LE and GE in sheep after Days 11 and 13 of pregnancy, respectively (Spencer et al., 1995). Further, PR expression is only detected in stroma and myometrium throughout most of gestation in the ovine uterus. The paradigm of loss of PR in uterine epithelia immediately prior to implantation is common to sheep (Spencer et al., 1995). Thus, regulation of endometrial epithelial function during the peri-implantation period must be directed by specific factors produced by PR-positive stromal cells in response to progesterone (Spencer and Bazer, 2004). In sheep, endometrial stromal cells express both fibroblast growth factor 10 (FGF-10) and hepatocyte growth factor (HGF) while endometrial epithelium and trophoctoderm express their respective receptors, FGF receptor 2IIIb (FGFR2IIIb) and *c-met* (Chen et al., 2000). The tunica intima of uterine blood vessels in sheep also expresses FGF-7, which acts via FGFR2IIIb. Mechanisms regulating these stromal-derived growth factors are not known.

Thus, progesterone is paradoxically involved first in suppressing and then inducing development of the endometrial luteolytic mechanism during the estrous cycle. The timing of PR down-regulation by progesterone appears to determine when the luteolytic mechanism develops in the endometrium. This hypothesis is supported by the finding that exogenous progesterone administration during metestrus decreased the interestrus interval in sheep and cattle (Garrett et al., 1988). Further, treatment of cyclic sheep with RU486, a PR antagonist, during the early luteal phase extended the interestrus interval (Morgan et al., 1993). PR antagonists prevent progesterone down-regulation of PR gene expression, thereby extending the period of PR expression (Spencer and Bazer, 2004).

## **2.7 Placentation and angiogenesis**

The placenta is the major organ through which respiratory gases, nutrients, and wastes are transported between the maternal and fetal systems. Its primary function is to supply the metabolic substrates necessary to support fetal growth. Besides, the placenta can play with the role of an autocrine, paracrine and endocrine organ which synthesizes a broad range of steroids and peptide hormones, which probably have a profound influence on growth and development of the fetus and utero-placenta, and perhaps even on their metabolism (Reynolds and Redmer, 1995). Additionally, the placenta can produce growth promoting substances e.g. Placental growth hormone (GH), prolactin like hormone (PRL-like hormone) and serve as a barrier that prevents migration of cells between maternal and fetal compartments (Gootwine, 2004).

Placental blood flow and vascular development are essential components of normal placental function and are critical to fetal growth and development. Normal fetal growth and development are important to ensure optimum health of offspring throughout their subsequent life course (Reynolds et al., 2006).

The importance of the placental circulation to fetal growth has been recognized since ancient times (Reynolds et al., 2005).

### **2.7.1 Structure and function of placenta**

The caprine placenta, characterized by the development of restricted areas of interdigitation between fetal and maternal tissues known as placentomes, is classified as cotyledonary. Histologically, the placentome is composed of the caruncular (maternal) tissue with its cripts filled with ramified projections of the chorion known as chorionic villi. In the placentome, the maternal blood circulation is separated from the fetal circulation by six tissue layers: maternal vascular endothelium, maternal connective tissue, syncytium, trophoblastic epithelium, fetal mesenchyma and fetal vascular endothelium, resulting that the caprine placenta is also classified as epitheliochorial. Wooding (1992) has proposed that the ruminant placenta be classified as synepitheliochorial due to the occurrence of migration of fetal cells through maternal-fetal junction and fusion of these cells with the maternal epithelial cells.

The maternal epithelium of the caruncular cripts persists throughout gestation as a syncytium, which is the result of binucleate chorionic cells fusion (Wooding, 1983; 1992). The trophoblastic epithelium is composed of columnar epithelial

intermixed with binucleate chorionic cells, which migrate through the maternal-fetal interface and fuse with the syncytial cells (Wooding, 1992). The binucleate cells correspond to 15 to 20 percent of the epithelial trophoblastic cells and approximately 1/7 of these cells are seen migrating to the caruncular maternal tissue (Wooding, 1983).

Although, the ruminant placentome has been a subject for studies by Wooding (1992) and Stegeman (1974) had studied some morphometric parameters and their relation to fetal development in sheep, there is no histometric evaluation of the main histological components of the caprine placentome, and no information about changes in its structural components throughout gestation. Moreover, the maturation process of the caprine placenta, including morphological changes at the end of gestation, is not well-known. In other ruminant species, mainly in bovinæ, it is known that many structural changes occur in the placentome at the end of gestation and these changes are related to the normal delivery of the placenta, including decrease in the number of binucleate cells and in the number of epithelial cells in the caruncular cripts (Williams et al., 1987).

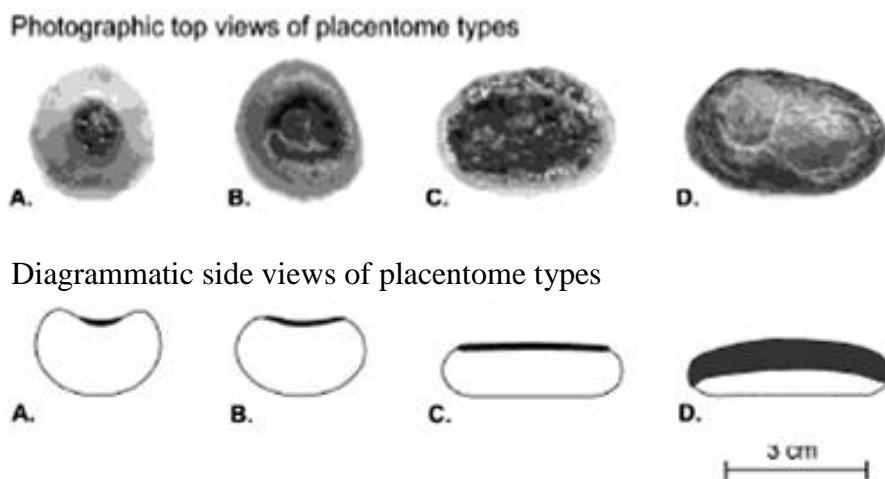
Stegeman (1974) showed that the average ratio between maternal and fetal tissues in the ovine placentome ranges from 0.4432 to 0.7293, and that the fetal connective tissue decreases, even with the increase in the degree of branching in the chorionic villi throughout gestation. Also, the degree of vascularization of the chorionic villi increases after the 10<sup>th</sup> week of gestation.

Ruminants comprise a large group of herbivores that include cattle, sheep, goats and deer. All of these animals, as described below, have cotyledonary placenta. The sheep placenta produces enough progesterone that by roughly day 70 the corpora lutea can be removed and pregnancy will not be interrupted. In contrast, luteal progesterone is required throughout gestation in cattle and goats because their placentae secrete much smaller quantities of progesterone. In reality, a large amount of progesterone is synthesized by the goat placenta, but most is converted to a biologically inactive pregnane before secretion (Bowen, 2000). The pattern of placental angiogenesis appear to differ between the cow and sheep, and therefore caution must be used when comparing the responses to altered nutrition during pregnancy between species (Vonnahme et al., 2006). In cows, low dietary protein in the first third of gestation

followed by increased protein in the second third of gestation enhanced placental development (Perry et al., 1999)

Ward et al. (2006) reviewed that ovine placentomes can be classified into 4 types using their gross morphological appearance. The fetal face of the placentome is defined by a thin hemophagous zone, where extravasated maternal blood lies between the maternal and fetal villi. This zone appears black and is inverted inside the bulk of the rounder A type placentomes. Only a small area of this zone is, therefore, visible on the external surface of the A type placentome (Figure 2.1). In the flatter D type placentomes, the hemophagous zone is everted and covers the entire top, fetal facing surface of the placentome (Figure 2.1). In between the A and D types are two more categories, the B and C types, which have intermediate degrees of hemophagous zone eversion (Figure 2.1). The A and B type placentomes predominate throughout gestation and, on average, account for 60% or more of the total number under normal conditions. The less common C and D categories occur with greater frequency late in gestation and in multiple pregnancies. These more everted placentomes also tend to be larger and heavier than the inverted A type category.

#### Photographic top views of placentome types



**Figure 2.1** Photographic top views and diagrammatic side views of the four types of ovine placentome

Source: Ward et al. (2006)

Fowden et al. (2008) reported that the factors affecting placental nutrient transfer capacity are as follows. 1) Placental size directly affects the capacity for nutrient transfer via changes in the surface area for transport and, when measured as placental weight, is positively correlated to bodyweight at term in a wide range of species (Fowden et al., 2008). 2) Morphology. Changes in both the gross morphology and ultrastructure of the placenta occur with increasing gestational age and in response to nutritional and endocrine manipulations. Many of these changes are interrelated and lead to alterations in surface area, vascularity, barrier thickness and cell composition of the placenta, all of which influence its transport characteristics. For instance, the reduction in binucleate cell population induced in the ovine placenta by early cortisol exposure may influence expansion of the feto-maternal syncytium, barrier thickness and placental hormone secretion.

### **2.7.2 Angiogenesis in placenta**

Angiogenesis and vascular transformation are important processes in the normal development of the placenta. Vasculogenesis is evident early in placental development when cells within the fetal villi differentiate into fetal endothelial cells and are organized into functional vessels. Vascular density in placental tissue is the indicator of placental growth. Vascularization consists of four processes: fragmentation of existing capillary basement membrane, proliferation (endothelial cell proliferation), migration (migration of endothelial cells from existing vessels), protease production and apoptosis.

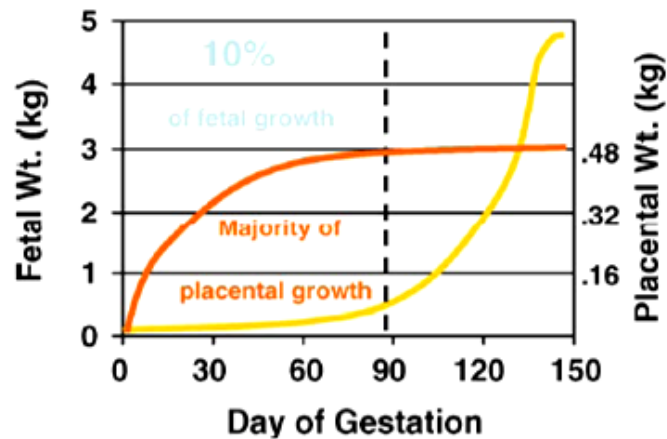
As early as day 18 of gestation in sheep, the allantoic membrane fuses with the chorion and the blood vessels of the newly formed chorioallantoic membrane will begin to proliferate (Reynolds and Redmer, 2001).

Borowicz et al. (2007) showed the patterns of vascular development in maternal (caruncular; CAR) and fetal (cotyledonary; COT) sheep placentas throughout the last two thirds of gestation, they found that the major angiogenic factors and their receptors were expressed from day 50 through day 140 of pregnancy in CAR tissues sheep placentas. Furthermore, vascularity increased 2-fold from day 50 through day 140 via relatively small increases in capillary number and 2- to 3-fold increases in capillary diameter. For COT, the increased vascularity resulted from a 12-fold increase in capillary number associated with a concomitant 2-fold decrease in capillary diameter. This large increase in fetal placental capillary number, which was due to increased branching,

resulted in 6-fold increases in total capillary cross-sectional area and total capillary surface, per unit of COT tissue.

Although fetal organogenesis is largely complete during early gestation, exponential growth of the fetus is limited to the last third of pregnancy (Figure 2.2), beginning around day 90 and ending around day 145 (term) in sheep. Thus, approximately 90% of fetal growth occurs during the last third of pregnancy. However, the majority of placental growth, at least in terms of placental mass, occurs during the first two-thirds of pregnancy, and the placenta achieves its maximum weight by day 90 in sheep (Figure 2.2). Thus, the fetus has obtained only 10% of its eventual birth weight by the time the placenta has reached its maximum size. Positive correlations between fetal and placental weight at term in a variety of species has been used to imply placental cause and fetal effect.

Definitive evidence that placental size is a major determinant of the subsequent fetal growth trajectory comes from a variety of invasive or extreme experimental models, which either impair placental growth or interfere with the uteroplacental blood supply. These include preparturient carunclectomy, maternal hyperthermia and utero-placental embolism. Alternatively fetal growth may be compromised by the inadequate availability of nutrients in the maternal circulation directly limiting fetal nutrient supply. It is, therefore, obvious that factors that influence placental growth will also affect fetal susceptibility to maternal dietary constraints later in pregnancy, thereby affecting birth weight, pregnancy success, and subsequent postnatal growth and development (Redmer et al., 2004).



**Figure 2.2** Relationship of fetal weight to stage of gestation in sheep

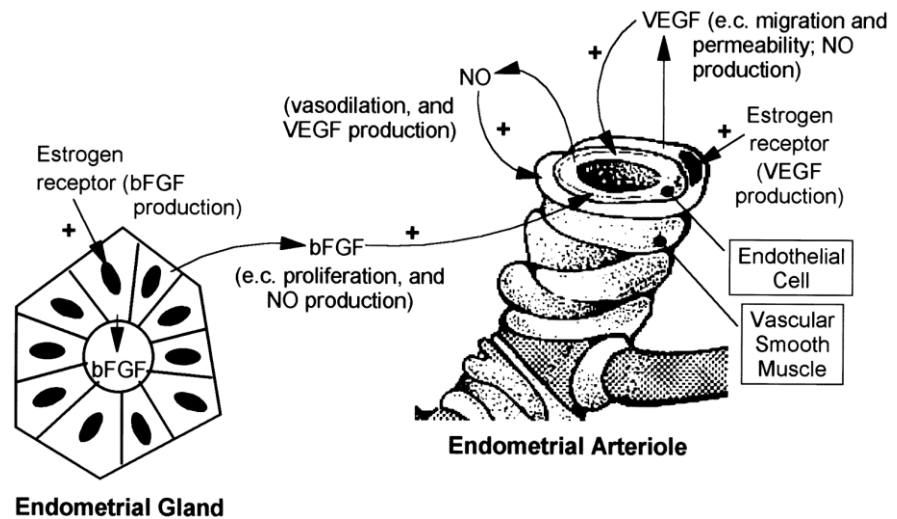
Source: Redmer et al. (2004)

### 2.7.3 Regulation of endometrial vascular function

Both VEGF and bFGF can stimulate endothelial production of nitric oxide (NO) by a variety of endothelial cells, including those from the uterine and fetal placental arteries. In addition, the uterine blood flow response to estrogen is probably mediated largely by NO, which can stimulate production of VEGF and bFGF. Moreover, endometrial VEGF is present primarily in arteriolar vascular smooth muscle and capillary pericytes, which is consistent with its localization to periendothelial cells in ovarian and other tissues. This is because endometrial microvascular ER also localizes to the vascular smooth muscle (Figure 2.3; Reynolds and Redmer, 2001).

ER protein is expressed in endometrial blood vessels in response to steroid treatment and also during early pregnancy. Estrogen receptor protein was upregulated in endometrial vascular smooth muscle by estrogen treatment of an ovariectomized ewe. In addition, ER protein was maintained in endometrial vascular smooth muscle during early pregnancy, in contrast to nonpregnant ewes in which vascular ER was dramatically reduced by day 14 of the estrous cycle. The vascular ER localized almost exclusively to the vascular smooth muscle of the endometrial arterioles. Endothelial NO synthase localizes to endothelial cells of endometrial arterioles and capillaries in ovariectomized, steroid-treated sheep and also in early pregnant sheep. These observations confirm other reports in sheep and humans and indicate that estrogen may mediate its effects on endothelial NO production indirectly via the vascular smooth muscle. In addition,

because endometrial ER also localize to the glandular epithelium, which produces bFGF, a role is suggested for estrogen-stimulated bFGF secretion in regulating endometrial vascular function (Figure 2.3; Reynolds and Redmer, 2001).



**Figure 2.3** Working model for regulation of endometrial (maternal placental) vascular function by VEGF and bFGF in sheep

Source: Reynolds and Redmer (2001)

VEGF gene expression was detected in the cotyledon, chorion, and amnion of the near-term ovine fetus. In these tissues polymerase chain reaction-amplified products corresponding to VEGF121, VEGF165, VEGF189, and VEGF206 were identified by ethidium bromide. A minor band of 1.7 kb was also found but only in the cotyledon and chorion. The abundance of messenger ribonucleic acid encoding VEGF was highest ( $P < 0.001$ ) in the cotyledon and lowest in the amnion. These findings suggest that vascular endothelial growth factor may play a role in the induction of angiogenesis and promotion of permeability in the microvessels that perfuse the placental and fetal membranes (Cheung et al., 1995).

VEGF mRNA was detected in the amniotic epithelium and the chorionic cytotrophoblastic cell layer. The increase in VEGF gene expression in fetal membranes as term approaches suggests that during fetal development VEGF may promote the vascularity and permeability of the microvessels which perfuse the fetal membranes, as

well as permeability of the amniotic membrane itself. Thus VEGF may participate in the regulation of amniotic fluid volume (Bogic et al., 2000).

Placental development requires adequate and organized interaction of vascular growth factors and their receptors, including vascular endothelial growth factor (VEGF) and placental growth factor (PGF). Both VEGF and PGF, acting through the tyrosine kinase receptors VEGFR-1 and VEGFR-2, have been implicated in playing a role in ovine placental vascular development. These alterations in growth factor and growth factor receptor mRNA expression, as a result of environmental heat stress early in placental development, could impair normal placental vascular development. Furthermore, alterations in VEGF, VEGFR-1 and VEGFR-2 mRNA expression, during the period of maximal placental growth, may contribute to the development of placental insufficiency, and ultimately intrauterine growth restriction (Regnault et al., 2002).

Vonnahme et al. (2008) studied the role of VEGF in increasing placental vascular density (PVD) in the pig. Hypoxia resulting from increasing fetal demands has been shown to increase VEGF gene expression in ovine placental cotyledons.

## **2.8 Factors affecting the pregnancy**

The tropical climate in Thailand is dominated by high temperature and humidity. Many factors affecting the pregnancy rate include internal (Breed) and external factor such as reproductive management, and nutritional management. For intrinsic factors, the heritability estimate for litter size was low at  $0.09 \pm 0.11$  goats to be  $1.40 \pm 0.05$  kids per litter in Thai native goat. However, many extrinsic factors can affect the pregnancy rate such as the following.

**2.8.1 Oxidative stress status:** In normal physiological conditions follicular fluid nitric oxide (NO) seems to be synthesized from granulosa cells by endothelial NO synthase, since in isolated human follicular cells at least 90% of cells are granulosa cells even though macrophages and lymphocytes. There are two major types of free radical species: reactive oxygen species (ROS) and reactive nitrogen species (NOS). Free radical Follicular fluid NO seems to be produced by either endothelial NO synthase or induced NO synthase. However, a positive correlation was determined between follicular fluid nitrate/nitrite levels and the follicular volume as well as the serum estradiol concentration. Follicular blood flow was found to be a better prognostic factor for predicting successful

outcomes with IVF than follicular NO levels. Follicular fluid NO levels were altered in patients with infertility associated diseases. NO follicular fluid levels were significantly higher in patients with endometriosis or hydrosalpinx compared to patients with tubal obstruction. No correlation was reported between the follicular NO levels and follicle maturity or follicle quality. Some studies have demonstrated the relationship between NO concentrations in follicular growth and programmed follicular cell death (apoptosis). Folliculogenesis involves the participation of both growth of the follicle and apoptosis. The role of nitric oxide in follicular atresia and apoptosis, in patients undergoing IVF was studied and it was found that the smaller follicles had significantly elevated percentage of apoptotic granulosa cells with nuclear fragmentation (Agrawal et al., 2005).

Low concentrations of NO may prevent apoptosis, however pathologically high concentrations of NO, as well as increased superoxide generation by NO synthase due to lack of arginine, may promote cell death by peroxynitrite generation. Nitric oxide involvement in various ovarian functions has been suggested. The presence of NO in the follicular fluid and the expression of NO synthase in follicles and corpus luteum.

Plasma concentration of NO was shown to increase in the follicular phase compared with the secretory phase and peaked at midcycle. Nitric oxide elicited a positive effect on women with poor ovarian response compared to controlled ovarian stimulation. Upregulated NO is harmful to implantation and pregnancy among patients with tubal factor infertility after controlled ovarian stimulation. Serum NO levels were elevated amongst nonpregnant patients with tubal or peritoneal factor infertility.

Follicular fluid NO level is not associated with maturity or quality of oocyte and no significant differences were seen in concentrations of NO of follicular fluid among large, medium, or small follicle size. Higher TNF- $\alpha$  concentrations (Tumor necrosis factor- $\alpha$ ) in follicular fluid correlated with poor oocyte quality. However, follicular fluid nitrite or nitrate levels were significantly lower in follicles containing mature oocytes that were fertilized compared with those that were not. Follicular NO has been reported to correlate negatively with embryo quality and the rate of embryo cleavage. The beneficial effects of NO donors in patients with intrauterine growth retardation (IUGR) and inhibition of preterm labor has been studied. Using a nitroglycerine (NTG) patch, which is a NO donor, did not significantly affect the final outcome in patients undergoing in-vitro fertilization. In addition, neither placebo nor the nitroglycerine patch improved the

flow resistance in the uterine artery. NO donors and elevated serum NO was associated with implantation failure resulting in decreased fertility.

**2.8.2 Maternal hypoxia:** There is an interaction between maternal hypoxia and alterations in placental and uterine blood flow, which could contribute to reduced nutrient transport to the fetus. At high altitude, less common iliac flow reaches the uterine artery. Despite an increase in uterine artery flow velocity, the uterine artery diameter is reduced, resulting in lower volumetric flow in late pregnancy. Studies of the placenta from high-altitude pregnancies have demonstrated that there is less remodeling of the uteroplacental arteries compared with those at moderate altitude. These studies suggest that physiological changes in response to high-altitude residence, which reduce blood flow to the fetoplacental unit, are detrimental for fetal growth (Murphy et al., 2006).

**2.8.3 Animal welfare and stress:** Housing affects the welfare of pregnant animals. Commonly expressed concerns include the following: 1) Animals should function well in the sense of being healthy and thriving; 2) animal should feel well, especially by prevention of serious pain, hunger, fear, and other forms of suffering and 3) animals should be able to live in a manner consistent with the nature of their species. In mammals, a wide range of challenges (eg. cold temperatures, disease, and aggression) may produce a stress response involving increased secretion of hypothalamic corticotrophin releasing factor (CRF; factor or hormone) and urocortin (UCN). Secretion of hypothalamic CRF causes 2 parallel effects; activation of the sympathetic nervous system (including secretion of catecholamines) and activation of the hypothalamic-pituitary-adrenal axis (HPA). Within the activated HPA, the pituitary secretes proopiomelanocortin, which is rapidly cleaved to release adrenal corticotrophin releasing hormone (ACTH),  $\beta$ -endorphin, and other peptides. Release of ACTH into the blood stream causes secretion of glucocorticoids.

The primary glucocorticoid secreted is cortisol. Elevation of cortisol within the blood negatively feeds back on hypothalamic CRF and ACTH to dampen the response of the HPA, unless the stressful event continues.  $\beta$ -endorphin may exert analgesic and cognitive effects that may help animals cope when stressed.

Stress-induced secretion of hypothalamic CRF (and associated intermediate hormones) has important peripheral physiologic effects. Secretion of CRF will cause increased heart rate and blood pressure, reduced gut motility, dilation of pupils, and of

CRF will cause increased heart rate and blood pressure, reduced gut motility, dilation of pupils, and mobilization of nutrients such as glucose. These physiologic responses help animals survive stressful experiences. Elevation of hypothalamic CRF and UCN and other neuropeptides (but generally not other hormones activated via the HPA) causes significant changes in animal behavior. Activation of CRF receptors results in behavior associated with fear and anxiety as well as stereotyped behavior. Stress also impacts immune system responses. In general, acute stress increases the number of neutrophils in the blood, while either not influencing or decreasing the relative number of circulating lymphocytes. The function of immune cells is also inhibited during stress. Examples include reductions in natural killer cell activity, lymphocyte response, and chemotaxis and phagocytosis of neutrophils. Furthermore, Romero et al. (1998) found a narrow association between elevated concentrations of maternal cortisol and the occurrence of abortion in goats free of *Brucella abortus*, *Brucella melitensis* and *Leptospira pomona*.

Some hormones, growth factor and healthy status may affect the pregnancy rate. Placental growth relates with fetal development and health of the offspring. Placental insufficiency is thought to be a key factor in many cases of intrauterine growth restriction which complicates about 6% of pregnancies in western countries. In ruminants, recent evidence supports the time of placental development and initial vascularization (between days 28 and 40) as a second period of significant loss. Recently, data have emerged leading to the suggestion that conceptus loss later in gestation is also significant and that variation in placental size and function may play a very important part in determining whether a conceptus survives. Understanding the molecular control of placental and fetal growth is essential to identifying diagnostic and therapeutic targets to improve pregnancy success (Roberts et al., 2008).

Several conditions are recognized as being associated with placental insufficiency and intrauterine growth retardation (IUGR) such as inappropriate nutritional management, genotype, heat stress, multiple pregnancy, etc. The changes in fetal and placental weights, uterine and umbilical blood flows and placental vascularity in various models of compromised pregnancy in sheep are shown in Table 2.1.

Triplet-born lambs are lighter at birth (Table 2.2) and display lower survival rates than their single- and twin-born counterparts. The lower birth weight of triplet-born lambs explains their lower survival rate as shown in the average birth weight of triplets study of Gootwine et al. (2007).

Serum P4 concentrations during late gestation increase as the number of fetuses increases. Total serum P4 on day 110 of pregnancy in ewes singleton-, twin- and triplet- born were  $4.45 \pm 0.24$ ,  $4.75 \pm 0.23$  and  $5.81 \pm 0.46$ , respectively (Figure 2.4).

**Table 2.1** Changes in fetal and placental weights, uterine and umbilical blood flows and placental vascularity in various models of compromised pregnancy in sheep

Model	Day of gestation	Fetal weight	Placental weight	Uterine blood flow	Umbilical blood flow	Vascularity
Overfed adolescent	130-134	↓20-28%	↓45%	↓36%	↓37%	↓31% (total capillary vol.)
Underfed adolescent	130	↓17%	NSE	-	-	↓20% (CAD in CAR)
Underfed adult	130-144	↓12%	-	↓17-32%	NSE	↓14% (CAD in CAR))
Adolescent vs adult	135	↓11%	↓29%	-	-	-
Genotype	130	↓43%	↓47%	-	-	↑36%
Heat-stressed adult	133-135	↓42%	↓51%	↓26%	↓60%	-
Multiple pregnancy	140	↓30%	↓37%	↓23%	-	↓30% (total capillary vol., COT)
High dietary Se	135	NSE	↓24%	-	-	↑20% (CND in COT)
Hypoxic stress	140	NSE	-	↓35%	-	↑ (CAD in CAR and COT)

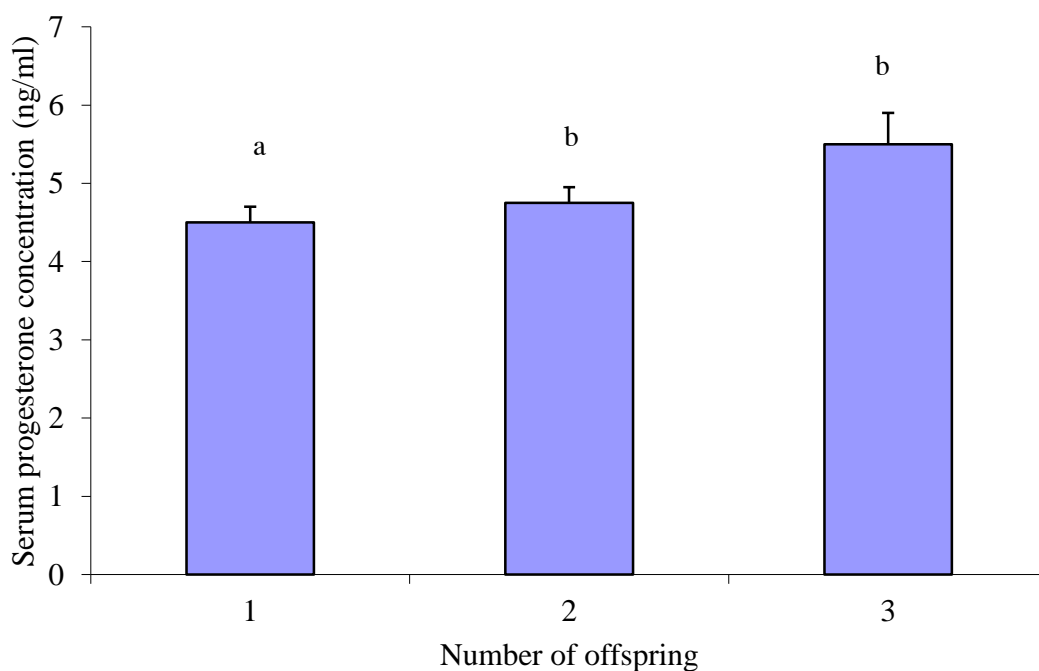
Source: Reynolds et al. (2006)

**Table 2.2** Least squares mean values for birth weight (BW) and growth rate (GR) up to 5 months of age of Afec-Assaf lambs

Litter size	No. of litters	BW (kg)	Growth rate (g/day) up to 5 months of age
1	1371	5.4 <sup>a</sup> ±0.03	291 <sup>a</sup> ±2
2	1540	4.4 <sup>b</sup> ±0.03	274 <sup>b</sup> ±2
3	777	3.6 <sup>c</sup> ±0.03	264 <sup>c</sup> ±2
4	248	3.0 <sup>d</sup> ±0.04	250 <sup>d</sup> ±2

<sup>a, b, c, d</sup> within a column, means with different letters differ significantly (P<0.05)

Source: Gootwine et al. (2007)

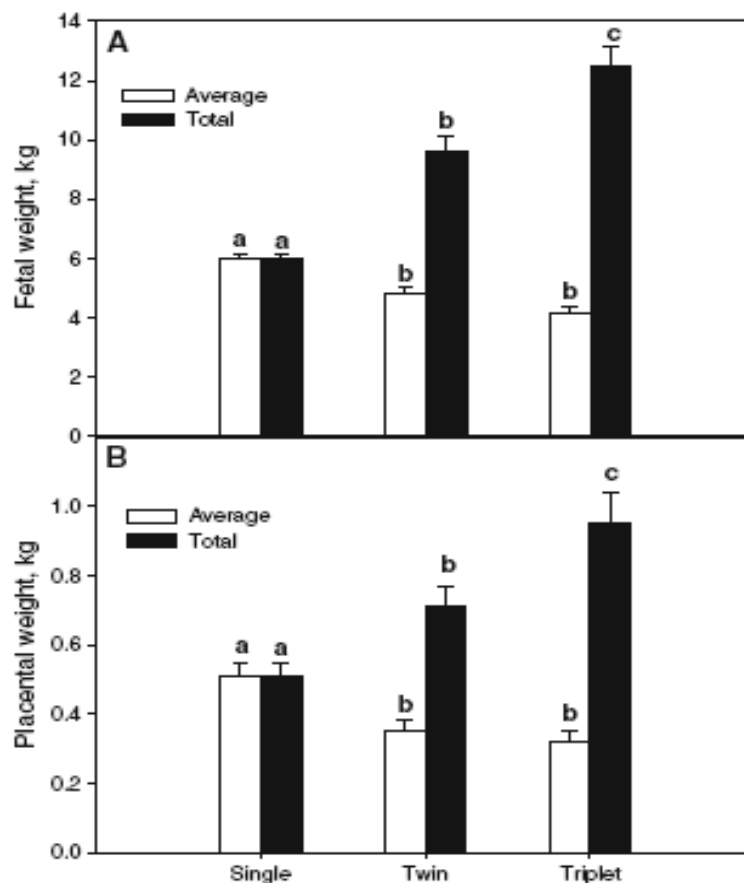


**Figure 2.4** Serum progesterone concentrations (ng/ml) during late gestation in mature ewes

Source: Butler et al. (1981)

## 2.9 Assessment of placental efficiency

Fetal and placental weights decreased ( $P < 0.05$ ) in twin and triplet pregnancies compared to singleton pregnancies. However, there was no difference in average fetal or placental weights between twin and triplet bearings in ewes. Total fetal and placental weight increased ( $P < 0.05$ ) as numbers of fetuses increased (Figure 2.5). However, placental efficiency, expressed as the ratio of fetal weight to placental weight, was similar for singleton, twin, and triplet pregnancies ( $12.2 \pm 0.9$ ,  $14.0 \pm 1.0$ ,  $13.4 \pm 1.6$ , respectively).



**Figure 2.5** Average and total fetal (a) and placental (b) weights from singleton, twin and triplet pregnancies on day 140 of gestation

a, b Means  $\pm$  SEM within a measure differ ( $P < 0.05$ ).

Source: Vonnahme et al. (2008)

Number of fetuses in the uterus did not affect capillary area density (CAD), capillary number density (CND), capillary surface density (CSD) or mean area per capillary (APC) in either caruncular or cotyledonary tissues (Table 2.3). Further, there was no difference in expression of Factor VIII, an endothelial cell marker, within a tissue for singleton, twin or triplet pregnancies (percentage area stained was  $2.1\pm 0.1$ ,  $1.9\pm 0.1$  and  $2.1\pm 0.2$ , respectively). When adjusted for total placental volume, total capillary volume per fetus in twin and triplet pregnancies tended ( $P=0.09$ ) to be less in the cotyledon, and was less ( $P=0.01$ ) in the caruncle, compared to singleton pregnancies (Table 2.3). Cotyledonary and caruncular weight per fetus was less ( $P<0.03$ ) in multiple compared to singleton pregnancies (Table 2.3).

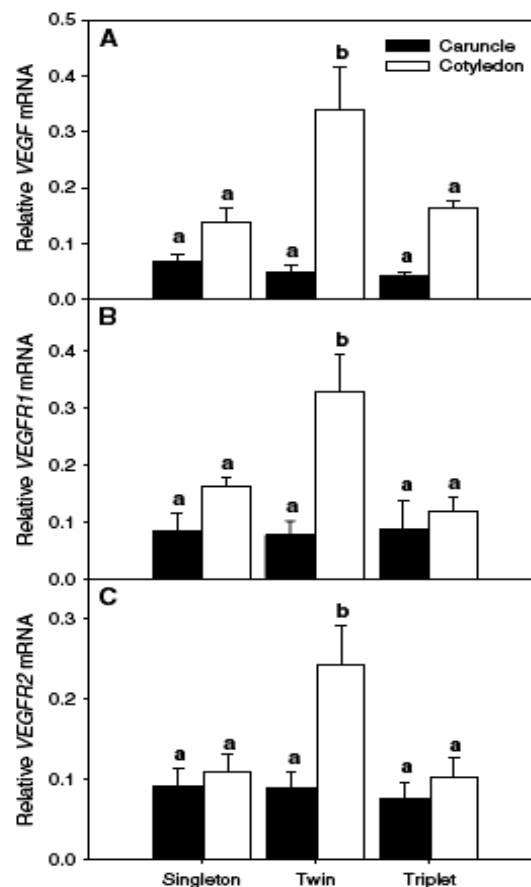
**Table 2.3** Caruncular and cotyledonary weights and capillary measurements from singleton, twin and triplet pregnancies

	Singleton	Twin	Triplet	P-value
Number of ewes	8	7	3	
Cotyledon				
Capillary area density	31.4±1.4	32.1±1.5	30.8±2.3	0.88
Capillary number density (no./ $\mu\text{m}^2$ )	3532±330	3472±352	2300±539	0.16
Capillary surface density ( $\mu\text{m}/\mu\text{m}^2$ )	8868±245	9047±261	8810±399	0.84
Mean area per capillary ( $\mu\text{m}^2$ )	100±16	103±17	145±26	0.34
Total capillary volume per fetus (ml)	55.8±0.6 <sup>a</sup>	35.5±0.7 <sup>b</sup>	25.2±1.0 <sup>b</sup>	0.09
Cotyledonary weight per fetus (g)	182.3±21.2 <sup>a</sup>	109.1±22.7 <sup>b</sup>	81.4±34.7 <sup>b</sup>	0.03
Caruncle				
Capillary area density	48.1±3.1	48.1±2.3	50.2±2.5	0.89
Capillary number density (no./ $\mu\text{m}^2$ )	1207±102	1421±174	952±127	0.17
Capillary surface density ( $\mu\text{m}/\mu\text{m}^2$ )	2284±78	2335±167	2081±109	0.49
Mean area per capillary ( $\mu\text{m}^2$ )	470±60	420±51	574±91	0.37
Total capillary volume per fetus (ml)	67.8±5.1 <sup>a</sup>	45.1±5.5 <sup>b</sup>	41.3±7.7 <sup>b</sup>	0.01
Cotyledonary weight per fetus (g)	140.7±9.8 <sup>a</sup>	95.1±10.6 <sup>b</sup>	82.2±15.0 <sup>b</sup>	0.01

<sup>a, b</sup> Means±SEM within row differ; Bold values are statistically significant P-values for specific measurements

Source: Vonnahme et al. (2008)

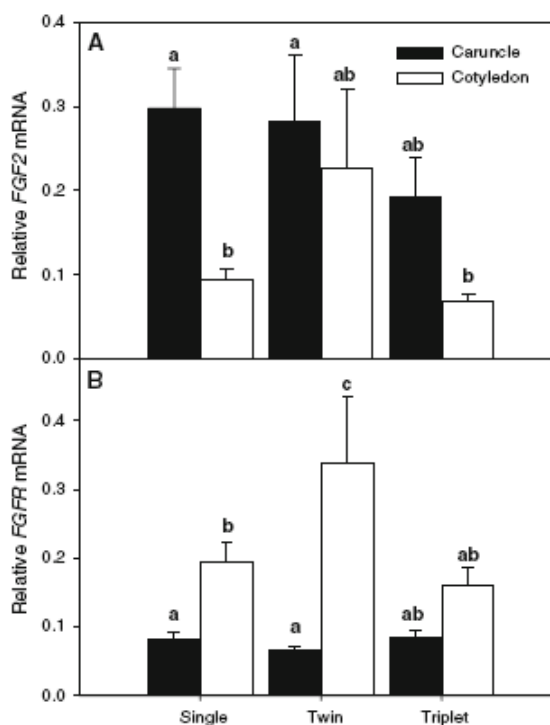
Caruncular VEGF, VEGFR1, and VEGFR2 mRNA expression were similar in singletons, twin or triplet pregnancies. Cotyledonary VEGF, VEGFR1, and VEGFR2 mRNA expression were enhanced ( $P<0.05$ ) in twin pregnancies compared to singleton and triplet pregnancies (Figure 2.6). While there was no difference of FGF2 mRNA expression in either caruncular or cotyledonary tissue across pregnancy types, expression of FGFR mRNA was greater ( $P<0.05$ ) in cotyledonary tissue from twin pregnancies than in cotyledonary tissue from singleton and triplet pregnancies (Figure 2.7). Expression of FGFR mRNA in caruncular tissue was similar for singleton, twin and triplet pregnancies (Figure 2.7).



**Figure 2.6** Caruncular and cotyledonary mRNA expression of VEGF (A), VEGFR1 (B), and VEGFR2 (C) from singleton, twin and triplet pregnancies on day 140 of gestation

<sup>a, b</sup> Means ± SEM within a tissue differ ( $P<0.05$ ).

Source: Vonnahme et al. (2008)



**Figure 2.7** Caruncular and cotyledonary mRNA expression of FGF2 (A) and FGFR (B) from singleton, twin and triplet pregnancies on day 140 of gestation  
<sup>a, b</sup> Means  $\pm$  SEM within a tissue differ ( $P < 0.05$ ).

Source: Vonnahme et al. (2008)

Expression of VEGF, VEGFR2, FGF2, and FGFR protein was detected in caruncular and cotyledonary tissues. However, the localization of VEGF, VEGFR2, as well as FGF2 and its FGFR protein in caruncular tissues differed from cotyledonary tissues. In the cotyledon, VEGF was localized in the walls of the major fetal arterioles and capillaries that were greater than 15  $\mu$ m in diameter and was not present in capillaries less than 10  $\mu$ m. In the caruncle, VEGF staining was present in the walls of most of the blood vessels. Positive staining for VEGFR2 was present in the walls of the caruncular blood vessels of all sizes, and in the cotyledon, was present only in binucleate cells. Positive staining for FGF2 was localized exclusively in the walls of caruncular blood vessels smaller than 15  $\mu$ m in diameter, whereas major caruncular blood vessels remained unstained, and no FGF2 staining was present in cotyledon. Positive, punctuate, scattered FGFR staining was detected in both caruncular and cotyledonary tissues. Protein

expression of VEGF was greatest ( $P < 0.001$ ) in placentomes from singleton, less from twin, and least from triplet pregnancies. The protein expression of VEGFR2 was similar ( $P = 0.94$ ) among placentomes of singletons, twins and triplets. While FGF2 protein expression tended to decrease ( $P = 0.10$ ) in placentomes from triplet pregnancies compared to placentomes from singleton and twin pregnancies, FGFR protein expression tended to be greater ( $P = 0.07$ ) in placentomes from twin pregnancies compared to singleton and triplet pregnancies.

### **2.10 Assessment of fetal growth**

Growth is one of the main attributes of living things and is such an obvious process that it hardly seems to justify any particular formal definition. The simple concept of growth meaning getting bigger is perhaps rather better than many of the complicated attempts to formalize something of such extraordinary complexity. In general, it is most helpful to use a descriptive word or phrase to qualify growth to identify the broad aspect with which one is concerned. For example, within one individual animal, one may speak about cell growth, organ growth, fetal growth, prepubertal growth, bone growth, chemical growth or negative growth and so on. In farm animals the main interest lies in the growth of specific parts of the animal such as bone, muscle, fat or the development of the mammary gland. These aspects of growth are readily appreciated and can be easily subjected to quantification either by weighing or by linear measurement. In this age of dramatic advances in the high technology of biochemistry and genetic engineering, it is helpful to remind ourselves of the biological significance of the size and physical form of animals (Lawrence and Fowler, 2002). Growth is a fundamental property of biological systems and it can be defined as body size per unit of time (Lawrence and Fowler, 2002).

Growth curves are used to describe the regular change generated by the live weight or some part of the animal with the age increasing which commonly is an S-type curve. Animal growth involves increase in size and changes in functional capabilities of the various tissues and organs of animals that occur from conception through maturity. The growth process includes increases in cell numbers (hyperplasia) and increases in cell size (hypertrophy). Growth performance of an animal is a phenotypic expression which is the outward expression of the animal genetic make up. Genetic factors have great influence on the performance of an animal. This explains the situation where within the

same breed or strain, individual variations in performance are common observations. Individuals that show superior performance should be identified and used in the genetic improvement programme (Chineke, 2001).

Fetal growth is dependent on genetic, placental and maternal factors. The fetus is thought to have an inherent growth potential that, under normal circumstances, yields a healthy newborn of appropriate size. The maternal-placental-fetal units act in harmony to provide the needs of the fetus while supporting the physiologic changes of the mother. Limitation of growth potential in the fetus is analogous to failure to thrive in the infant. The causes of both can be intrinsic or environmental (Peleg et al., 1998).

### **2.11 Summary**

The research focused on and investigated the application of assisted reproductive technology (ART) to induce estrus synchronization, superovulation, and ovulation using progestins and gonadotropins (PMSG and FSH) to produce a large number of good oocytes suitable for in vitro production. Furthermore, there were studies on fetal and maternal growth pattern during early gestation using ultrasonography, to develop the equation of fetal growth between single, twin and triplet pregnancies. Additionally, there were studies on the effect of maternal age on placental and fetal growth in the Thai native goat. Knowledge of this study can be applied to improve the health of offspring in Thai native goats and reproductive management.