

CHAPTER V

**EXPERIMENT III EFFECT OF MATERNAL AGE AND WEIGHT
ON NUMBER OF FETUSES, PLACENTAL GROWTH AND
FETAL DEVELOPMENT IN THAI-NATIVE GOAT**

5.1 Experiment 3.1: Study on fetal growth during early gestation in Thai-native goat using ultrasonography

5.1.1 Introduction

Early gestation is a critical period for placentomal growth, differentiation, and vascularization, as well as fetal organogenesis (Vonnahme et al., 2008). Gootwine et al. (2007) suggested that regulation of fetal growth involves multidirectional interactions between the mother, the placenta and the fetus. Various methods have been used to diagnose pregnancy in sheep. These methods can be classified as less practical such as the management method (non-return to estrus), abdominal palpation and ballotment, palpation of the caudal uterine artery, laparotomy, peritoneoscopy and rossete inhibition test reviewed by Ishwar (1995), and the most practical methods such as radiography, rectal abdominal palpation, hormonal assays, pregnancy protein assays and ultrasonography.

In 1980, B-mode ultrasonography was introduced in the veterinary field and used for pregnancy diagnosis in mare (Driancourt and Palmer, 1982) and then received large acceptance for diagnosing pregnancy in all domestic animals (Kahn, 1992). Transrectal ultrasonography has been recommended as a simple, rapid and practical method for early pregnancy diagnosis in sheep (Buckrell et al., 1986; 1988). However, the accuracy of this technique is greatly variable (Gearhart et al., 1988, Garcia et al., 1993). Predictions of the number of fetuses would allow appropriate nutritional management of the ewes in late gestation that will prevent pregnancy toxemia, minimize prelambling feeding costs, optimize birth weight, weaning weight and survivability of lambs and reduce the incidence of dystocia (Gearhart et al., 1988). In addition, the accurate information on the stage of gestation would be useful to dry off lactating females at an adequate period and to monitor the females near term (Doize et al., 1997).

There is a lack of information about fetal and maternal growth in the Thai-native goat. B-mode ultrasonography is an accurate, rapid and safe method for diagnosing pregnancy in small ruminants. Transrectal or transabdominal approaches can be used with a nearly 100% accuracy rate (Doize et al., 1997). In a previous study, McDonald et al. (1988) and Martinez et al. (1998) reported that ultrasonic measurement of crown-rump length was useful in predicting the age of the goat embryo.

5.1.2 Materials and Methods

1) Animals and Ethics

All experimental procedures were conducted under protocols approved by the Khon Kaen University Animal Care and Use Advisory Committee (AEKKU 40/2552). Eleven Thai native goats had average age and weight of 31.7 ± 4.1 months, and 28.28 ± 2.17 kg, respectively. Animals were fed with concentrate (16% CP) and ad libitum feeding of fresh grass to meet the requirements in accordance with NRC (1981). Clean water and mineral block were provided for animals ad libitum. Animals were vaccinated against foot and mouth disease (FMD), hemorrhagic septicemia (HS) and brucellosis, according to the standard farm requirement of the Department of Livestock Development and the University farm.

2) Estrus synchronization and Ultrasonography

Estrous synchronization was accomplished by using 60 mg MAP for 14 days. Thereafter, the animals were placed with the fertile male. Pregnant animals were individually kept in the pen throughout the gestation. Does were weighed and examined by real time ultrasonography scanner model HS-2000 (HONDA ELECTRONICS, Japan) with 5-7.5 MHz linear array transducer. The Crown-Rump Length (CRL) during days 28 to 49 of gestation was measured as described by Gonzalez-Bulnes et al. (1998). The data were expressed as means \pm SEM. Fetal and maternal growth were estimated by the growth equations according to Redmer et al. (2004).

3) Maternal weight

Does were initially weighed and then every 7 days interval throughout the experimental period.

5.1.3 Results and Discussion

1) Fetal growth

From the results of experiment 3.1, the fetal growth equations as determined by the CRL for single, twin and triplet pregnancies were $y_1 = 5.5287e^{0.3477x}$ ($r^2 = 0.9714$), $y_2 = 5.3115e^{0.5304x}$ ($r^2 = 0.9626$) and $y_3 = 7.2716e^{0.3778x}$ ($r^2 = 0.9237$), respectively (Figure 5.1). The growth pattern from the study according to Gonzalez-Bulnes et al. (1998), demonstrated that fetal growth in terms of CRL measurement during days 19-48 of gestation in Menchaga fetuses had exponential pattern. However, Martinez et al. (1998) evaluated the CRL on day 19 of gestation in Anglo-Nubian goats as 5.3 ± 0.3 mm, reaching 34.2 ± 0.6 mm on Day 40. The CRL from day 19 to 38 of pregnancy was best represented by a linear regression ($Y = -2.23 + 0.13X$; $r^2 = 0.94$). However, the growth of the fetus during early pregnancy exhibited the exponential growth, with twin pregnancy having the greatest growth rate, followed by triplet and single pregnancy. Gonzalez-Bulnes et al. (1998) reported no significant differences have been observed between single or twin pregnancies in Menchaga ewes.

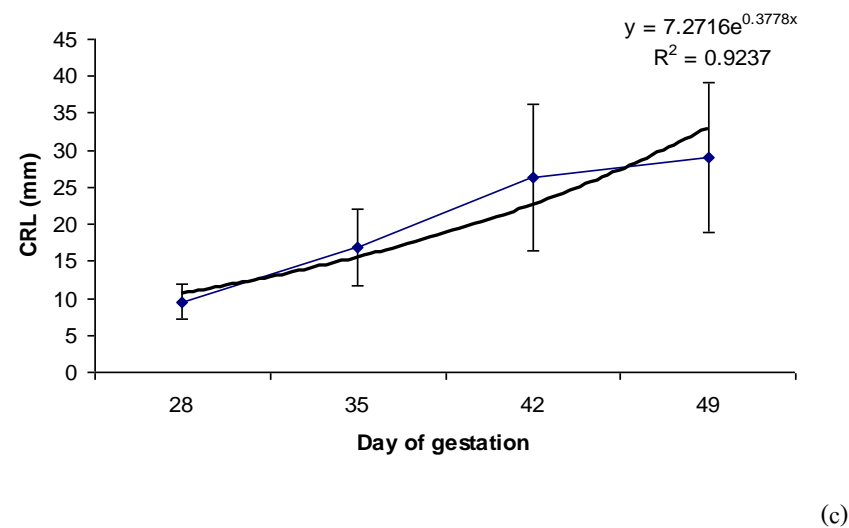
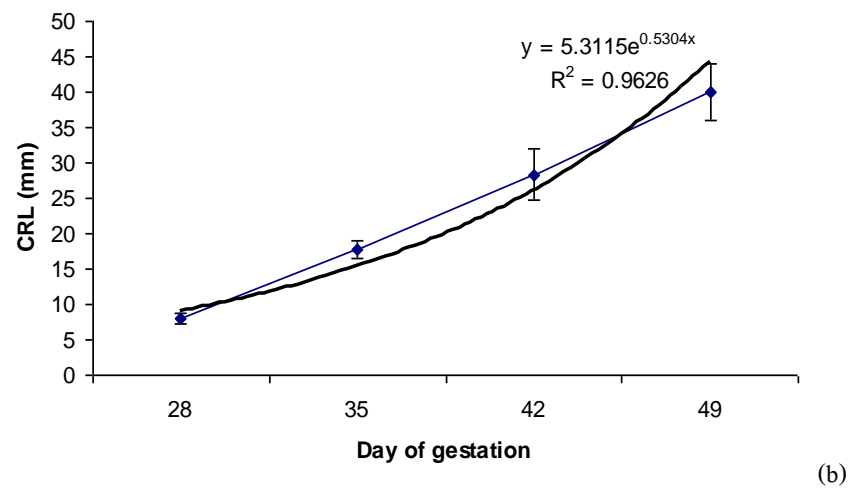
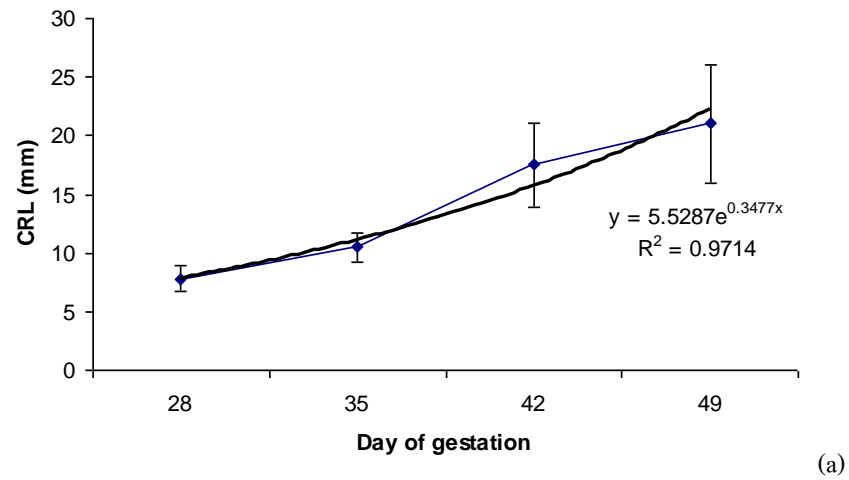


Figure 5.1 Relationship between day of gestation and crown-rump length from single (a), twin (b), and triplet (c) pregnancy in Thai-native goat

2) Maternal growth

Maternal growth for single, twin and triplet pregnancy increased gradually with the linear equation of $y_1 = 0.75x + 25.138$ ($r^2 = 0.9597$), $y_2 = 0.855x + 28.208$ ($r^2 = 0.9889$) and $y_3 = 0.8x + 31$ ($r^2 = 0.9143$), respectively. The results from this study are similar to the study of Wallace et al. (2006), who concluded that the maternal growth during early pregnancy between single, twin and triplet in ewe pregnancy were slightly increased. The average early pregnancy diagnosis was 25.0 ± 1.4 days, confirmed with the embryonic vesicle located in utero-cranial to the bladder (Gonzalez-Bulnes et al. 1998) and the fetal heart beat (Doize et al., 1997).

5.1.4 Conclusions

Fetal growth during early gestation exhibited the exponential growth, in which twin pregnancy had greatest growth rate, followed by triplet and single pregnancy, whereas maternal growth exhibited the linear growth as determined by ultrasonography.

5.2 Experiment 3.2: Effect of maternal age and weight on number of fetuses, placental growth and fetal development in Thai-native goat

5.2.1 Introduction

Pregnancy during human adolescent life (19 years of age) annually accounts for up to one-fifth of all births worldwide and carries an enhanced risk of poor obstetrical outcomes, including premature delivery, low birth weight, neonatal and infant mortality, and maternal death. Pregnant adolescents are additionally vulnerable to both inadequate and excessive gestational weight gains, which may interact with their growth status or have an impact on pregnancy outcome (Wallace et al., 2010). Wallace et al. (2006) report that maternal live weight and BCS in adolescent pregnancy ewes with moderate and high nutrition levels have differences, because in the high nutritional group have increased body fat deposition which influences for high growth rates and nutrient partitioning to the gravid uterus, causing shortened gestation, high abortion incidence, decreased placental and fetal growth and low lactation performance. In contrast in the well nourished adolescent pregnancy, growth of the maternal body is prioritized at the expense of the nutrient requirements of the gravid uterus and mammary glands. Redmer et al. (2004) showed that the placental growth restriction is associated with high levels of insulin and IGF-I while progesterone is at a low level.

This alteration in the normal hierarchy of nutrient partitioning is attributed to a competition for nutrients between the maternal body and her gravid uterus and has been partly replicated in a highly controlled overnourished sheep paradigm. Using this paradigm, we have consistently shown that overfeeding of the young singleton-bearing adolescent throughout pregnancy to promote maternal growth and high gestational weight gains similarly results in the premature delivery of low-birth-weight lambs (Redmer et al., 2004) when compared to control-fed adolescents of equivalent gynecological age. Furthermore, Wallace and co-workers demonstrated that defects in early placental development (namely reduced cellular proliferation, impaired angiogenesis and vascular development, and attenuated uterine blood flow) compromise the placental growth trajectory and are the primary limitations to fetal growth in the rapidly growing adolescent sheep (Wallace et al., 2010). Thus, by late gestation, and in spite of the ready availability of nutrients in the maternal circulation, the small size of the placenta and the resultant attenuation of uteroplacental blood flows and nutrient uptakes severely limit fetal nutrient supply, resulting in major fetal growth restriction at term (30%–40% reduction relative to controls).

The placenta is the organ that transports nutrients, respiratory gases and wastes between the maternal and fetal systems. The development of its vascular beds is essential to normal placental function, and thus in supporting normal fetal growth (Reynolds et al., 2005). Vascular growth of maternal (caruncular; CAR [specifically, the caruncular portion of the endometrium]) and fetal (cotyledonary; COT [specifically, the cotyledonary portion of the chorioallantois]) placental tissues begins early in pregnancy and continues throughout gestation (Borowicz et al., 2007).

Inadequate vascular growth in placental tissues during early pregnancy may be associated with inadequate uterine and umbilical blood flow, which directly affects transport of nutrients to the embryo and fetus. The consequences of inadequate placental vascular development include compromised implantation, spontaneous abortion/ embryonic loss, defective formation of the placenta, and altered fetal growth and development resulting in intrauterine growth retardation (IUGR) potentially leading to reduced lifelong health and productivity of the offspring (Wallace et al., 1996, Reynolds et al., 2005; Grazul-Bilska et al., 2010).

Maternal age has a large influence on fetal and placental growth. However, to what degree is this effect due to maternal immaturity or to numerous confounding factors (Wallace et al., 1996). Wallace and co-workers have consistently shown a 30 to 40% reduction in fetal and placental weights late in gestation in adolescent ewes that were overfed throughout pregnancy. During pregnancy, the adolescent ewe group has a high concentration of, insulin, blood glucose, IGF-I, Tri-iodothyrosine (T3) and thyrosine (T4). These results suggest the anabolic drive to maternal tissue synthesis is established during early gestation at the expense of placental growth (Wallace et al, 1996; 1997).

During pregnancy, nutrients are generally partitioned to various tissues of the maternal body in priority order according to their metabolic rate. Thus, as nutrients become limited, tissues with lower metabolic rates and hence reduced priority, will receive quantitatively less nutrients. Since highly metabolically active tissues receive greater rates of blood flow than less metabolically active tissues, the rate of blood flow becomes the limiting factor for nutrient partitioning to specific tissues. In normal adult pregnancy, the gravid uterus receives a very large proportion of total cardiac output resulting in a high priority status for partitioning of nutrients. However, the hierarchy of nutrient partitioning in adolescent pregnancy shifts from what is seen in the adult to a higher priority status for nutrient partitioning toward maternal tissue growth and fat deposition. Therefore, the differences between adults and adolescents in the hierarchy of nutrient partitioning has allowed for the development of unique animal models for studying the mechanisms by which nutrient intake affects overall fetal growth and development (Redmer et al, 2004).

Vascular endothelial growth factor is a potent angiogenic growth factor. The role of VEGF also known as vascular permeability factor (VPF), has been well established as a stimulator of angiogenesis primarily through its mitogenic permeability and migration-stimulating activity on endothelial cells (Navanukraw, 2003).

Additionally, pregnant adolescent ewes fed a high energy or maintenance diet showed a significant reduction in placental expression of mRNAs for Vascular Endothelial Growth Factor (VEGF), Angiopoietin-1 (ANGPT1) and Angiopoietin-2 (ANGPT2) in the placenta at mid-gestation, before any reduction in fetal or placental size was observed (Reynolds et al., 2005). In the same experiment, although placental

expression of the receptors for VEGF and ANGPT1 and ANGPT2 also were evaluated, only FLT1 mRNA was reduced (Reynolds et al., 2005).

In another experiment, capillary size was increased and capillary number density was reduced in maternal and fetal placental tissues of overfed adolescent ewes on day 130 of gestation (Redmer et al., 2004). These effects of altered placental growth and angiogenesis result in intrauterine growth retardation (Wu et al., 2006), which can be defined as impaired growth and development of the mammalian embryo/fetus or its organs during pregnancy.

The objectives of this experiment were to establish growth equations by investigating the fetal and maternal growth during early gestation in Thai-native goats using ultrasonography and to compare the number of fetuses, placental efficiency, mRNA expression of VEGF165 in placental tissue and blood concentration of cortisol, and progesterone between adolescent and normal pregnancies in Thai-native does.

5.2.2 Materials and Methods

1) Animal Ethics

The experiment was approved by the Animal Ethics Committee of Khon Kaen University (No. AEKKU 40/2552). The study was conducted at the experimental farm, the small ruminant unit, Department of Animal Science, Faculty of Agriculture, Khon Kaen University during November 2009-January 2011. The university is located at 102 degrees east longitude and 16 degrees north latitude with a tropical climate.

2) Animals and Treatments

Eight adolescent nulliparous Thai-native goats (7 months and 17.83 ± 0.39 kg) and eight adult nulliparous Thai-native goats (24 months and 29.75 ± 1.65 kg) were used for this study. The does exhibited at least 2 normal estrous cycles. Heat detection was recorded using a vasectomized buck daily, at 6.00 A.M. and 5.00 P.M. followed by Gordon (1997) and Cognie (1999). Animals were housed in freestall barns located at the small ruminant unit, Department of Animal Science, Faculty of Agriculture, Khon Kaen University. Animals were fed a ration of hay and fresh grasses twice daily for ad libitum consumption to meet or exceed requirements for growth and pregnancy (NRC, 1981).

3) Estrous synchronization and mating system

Estrous synchronization was accomplished by using 60 mg MAP for 14 days. Thereafter, the animals were placed with an intact male during estrus for natural mating.

4) Data collection

(1) Maternal weight

Estrous does were initially weighed and then at every 7 day intervals throughout the experiment period. Average daily gain was calculated as the difference in weight between the final and initial weights divided by the interval in days from the dates the initial and final weights were taken, i.e., $ADG = \text{Weight gain} / \text{interval in days}$.

(2) Conception rate and pregnancy rate

Pregnancy diagnosis were performed by real time ultrasonography scanner model HS-2000 (HONDA ELECTRONICS, Japan) on day 21 of gestation as described by Gonzalez-bulnes et al. (1998). Visualization of fluid-filled uterine horn and the presence of a conceptus were used as positive indicators of pregnancy at 21 d after mating using an ultrasonography machine equipped with a transrectal 5-7.5 MHz linear array transducer. The number of does diagnosed was used as the conception rate.

(3) Abortion rate

The number of aborting does was divided by the total number of goat.

(4) Number of placentomes

Placentomes were counted and weighed and obtained from each group on days 65 and 130 after mating (gestation length in Thai-native goat is approximately 148 days). The number of fetuses were recorded as single, twin or triplets. Four representative placentomal types were classified as described by Ward et al. (2006).

(5) Vascularity measurement

Placentomes were counted and weighed and obtained from each group on days 65 and 130 after mating. Then, placentomes were cut cross sectionally into several pieces (1-2 mm/piece) and were fixed in Carnoy's solution (absolute ethanol: chloroform: glacial acetic acid, 6:3:1) for 2 h at room temperature. Placentomal pieces were then stored in 70% ethanol until further processing. Fixed placentomal pieces were

dehydrated by using a graded series of ethanol, cleared with a histological clearing agent (Histo-clear[®], National Diagnostics, Atlanta, GA), paraffin embedded. Tissue sections 6 μm thick were stained with hematoxylin-eosin and PAS for microscopic examination and mounted onto glass slides as previously described (Grazul-Bilska et al., 2007). Vascularity was determined in histological sections of each placentome. Then, hematoxylin and periodic acid-Schiff's (H&E and PAS) reagent staining was performed as previously described (Borowicz et al., 2007). Photomicrographs were taken at 200x magnification. Vascularity was determined by capillary area density (CAD), capillary number density (CND) and area per capillary (APC). The CAD, CND and APC were determined by using an image analysis system (Motic Image Plus 2.0, Olympus, JA) as described previously (Guntaprom, 2010; Nutthakornkul, 2011). For each placentome, 8 randomly chosen fields (130,000 μm^2 per field) were evaluated separately for CAR, COT and total tissue (CAR and COT) area. The data were expressed as mean percentage \pm SEM of the total area that exhibited vessels within each tissue area.

(6) Serum progesterone concentration

7 ml blood samples were taken on days 0, 28, 56, 84, 112, and 140 of gestation by jugular venipuncture to determine serum progesterone. Samples were allowed to clot at room temperature and were centrifuged at 3,000 g for 20 min. within 1 h after collection. The serum was stored at -20 °C until further processing. Serum progesterone concentrations were determined by competitive ELISA (Crane et al., 2006). Goat anti-mouse IgG (H+L) was made in mouse by using a P4-horse radish peroxidase conjugate. Intraassay coefficient of variation of 4.65% and assay sensitivity was 0.025 ng/ml.

(7) Serum cortisol concentration

5 ml blood samples were collected from goats by jugular vein puncture for cortisol determination prior to feeding and weighing on days 28, 84 and 130 of gestation. Samples were allowed to clot at room temperature and were centrifuged at 3,000 g for 20 min within 1 h after collection and then transported to the laboratory. Serum cortisol concentrations were analyzed by the Laboratory center of Medical Science, Khon Kaen University.

(8) mRNA VEGF quantification

Placentomes from the gravid uterine horn were obtained from each does on days 65 and 130 after mating. The placentomes were placed into a 60 mm petri dish washed with phosphate buffer solution (PBS) and separated into two compartments eg. CAR and COT. The CAR and COT tissue were collected and transferred to microtubes. All tubes were snap frozen for evaluation of VEGF165 gene expression using a real-time reverse transcriptase polymerase chain reaction (real-time RT PCR).

Fluorescent, real-time quantitative RT-PCR was used to determine differences in mRNA expression of VEGF165. Total RNA was extracted from CAR and COT tissues by the High Pure RNA Tissue Kit[®] (Roche Applied Science, Germany). Optical densities at 260 and 280 nm were measured to determine the quantity and purity of RNA samples. An amount of 2 mg total RNA was subjected to reverse transcription with Transcriptor High Fidelity cDNA Synthesis Kit[®] (Roche), according to the manufacturer's protocol, using 2 µl random hexamers. Primers (Table 5.4) for quantitative RT-PCR were made with Primer Quest software (Integrated DNA Technologies, Coralville, IA, USA) according to the manufacturer's restrictions. Each PCR reaction (total volume of 50 µl) consisted of 5 µl cDNA template, 25 µl Faststart SYBR Green Master, 1.5 µl forward primer, 1.5 µl reverse primer, and 19 µl water (PCR grade). Thermal cycling conditions were as follows: 10 min at 95 °C for pre-incubation, 40 cycles of 20 s at 95°C for denaturing, 20 s at 55 °C for annealing, and 45 s at 72 °C for extension, followed by a ramp from 65 °C to 95 °C over 1 min to determine the melting curve. Quantification of caprine VEGF165 gene, primer and condition, was previously published by Kawate et al. (2003). The caprine VEGF165 amplified by RT-PCR. The nucleotide sequences are shown in Table 5.1.

Table 5.1 Nucleotide sequences of forward and reverse primers to amplify each fragment for cDNAs encoding caprine VEGF165

| Primer orientation | Nucleotide sequence | Accession number |
|---------------------------|----------------------------------|-------------------------|
| Forward primer | 5' – GTGCGGGGGCTGCTGTAATGACG- 3' | AY114352 |
| Reverse primer | 5' - GGTGGTGGCGGCGGCTATGG- 3' | AY114353 |

5) Statistical Analysis

All data such as initial body weight, body weight on day 63 of gestation, difference in body weight, and average daily gain, were analyzed using the procedure Means of SAS (2001). Placentomal type ratio between adolescent and adult pregnancies on day 65 and 130 of gestation in Thai-native does were statistically analyzed by using the student's *t*-test (Steel et al., 1997).

5.2.3 Results and Discussion

1) Maternal growth during gestation

The average maternal initial BW in the adult does group (29.75 ± 1.65 kg) was greater than in the adolescent does group (17.83 ± 0.39 kg, $P < 0.01$). The average maternal BW on day 63 of gestation in the adult does group (33.65 ± 1.98 kg) was greater than in the adolescent does group (19.74 ± 0.68 kg, $P < 0.01$). The average daily gains (ADG) was not statistically different between adult (0.11 ± 0.03 kg) and adolescent does (0.05 ± 0.02 kg). The ADG of Thai-native goat as previously reported by Sripongpun et al. (2001) was lower (39.0 ± 0.6 g/day/head) than both groups in the present study. In addition, the ADG of this study was higher than the Laos-local goat at 44.6 ± 1.6 g/day/head in the growing period (Phengvichith and Ledin, 2007). Does in the experiment had the same BCS, although the initial BWs was different ($P < 0.01$). At day 63 after mating, BWs of adult and adolescent does was not different. The ADGs of pregnant Thai-native does was similar to pregnant ewes. Wallace et al. (2000) reported that pregnant ewes receiving moderate nutrient increased average maternal ADG at 90 g/day during 100 day of gestation.

Table 5.2 Average daily gain of adolescent and adult pregnant goats during first 63 days of gestation

| Measurements | Adolescent | Adult | P-value |
|---|------------|------------|---------|
| Initial body weight (kg) | 17.83±0.39 | 29.75±1.65 | <0.01 |
| Body weight on day 63 of gestation (kg) | 19.74±0.68 | 33.65±1.98 | <0.01 |
| Body weight gain (kg) | 1.91±0.70 | 3.90±1.02 | 0.07 |
| Average daily gain (kg/day) | 0.05±0.02 | 0.11±0.03 | 0.07 |

Maternal growth for adolescent and adult pregnancies increased gradually with the linear equation of $Y1 = 0.408x + 17.28$ ($r^2 = 0.969$), and $Y2 = 0.822x + 28.63$ ($r^2 = 0.964$), respectively presented in Figure 5.2.

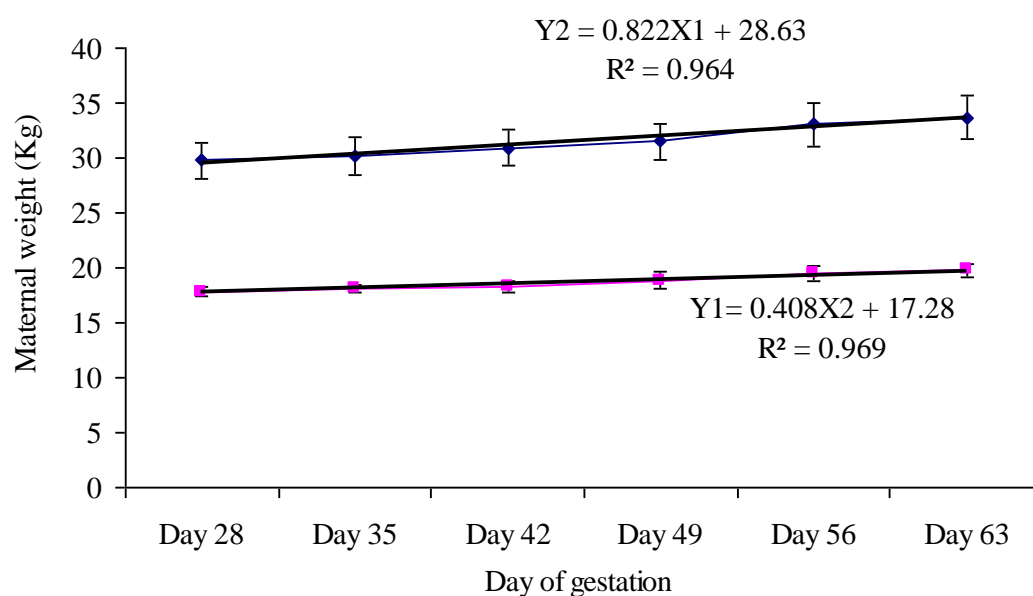


Figure 5.2 Maternal weight during 28-63 of gestation between adolescent (Y1) and adult (Y2) pregnancies

2) Conception rate and pregnancy rate

Conception and pregnancy rate between adult and adolescent groups were 100% after estrus synchronized with MAP sponge for 14 days. Estrus detection was performed using a vasectomized buck. A buck was introduced in the herd for breeding. Pregnancy was confirmed by ultrasonography after 53 days of sponge removal (Imasuen and Ikhimioya, 2009). After mating, estrus was induced in 16/16 goats (100%) and the interval from sponge removal to estrus was 72 h. There were no differences in conception rate between the two groups (100%).

In an animal farm, the conception rate is related to fertility, estrus detection accuracy, semen fertility and mating technique. The conception rate is estimated by multiplying the percent efficiency for each of the factors. First, note that when efficiencies are all 90% or higher, the maximum conception rate that can be expected is 65-70%. Thus when evaluating conception rate and setting goals, it is sensitive to be realistic (Smith, 1982). It is extremely difficult in the long run to get more than 70 pregnancies out of every 100 straws of semen that are used. Conception rates of 60 to 70% using AI are excellent in cows. For goat, Mellado et al. (2006) reported that the warmest season favoured conception rate (70% in spring, $P < 0.01$) as compared to cooler seasons (62% and 64% for summer and autumn, respectively). The pregnancy rate of adult does in the present study was higher (75%) than that previously reported by Imasuen and Ikhimioya (2009). Pregnancy rates of goats receiving MAP for 11 days at day 53 after AI were 52.6%.

3) Abortion rate

In adult pregnancies, 2 does subsequently reabsorbed their fetuses between the ultrasound examinations on day 45 and 75 of gestation. In adolescent pregnancies, 6 does subsequently reabsorbed their fetuses on day 45 and 75 of gestation. The abortion rate of adult pregnancies at 25% (2/8) was lower than for adolescent pregnancies at 75 % (6/8).

4) Number of placentomes

The morphologic type of placentome was classified following the method previously described by Vatnick et al. (1991) and was based on the placentome appearance, as follows: 1) caruncular tissue completely surrounding the cotyledonary tissue (type A), 2) cotyledonary tissue beginning to grow over the surrounding caruncular

tissue (type B), 3) flat placentomes with cotyledonary tissue on one surface and caruncular tissue on the other (type C), and 4) everted placentomes resembling bovine placentomes (type D). Intermediate types should be classified to the upper level such as intermediate type B-C can be classified into C type. Type and percentage of placentomes on day 65 and 130 of gestation in Thai-native does are shown in Table 5.3. Placentomal shape changes during placental development. The concave placentomes is characteristic of the *Caprini*. The caprine placenta is comprised of four distinct placentomal types which progressively develop from type A, through types B, C and D. It has been hypothesized that this progression of placentomal types increases placental efficiency (Ward et al., 2006). The placentomal type of Thai-native does can change the shape from A- to D- type as day of gestation advanced and the ratio of types A+B/C+D may used to predict the placental efficiency during gestation. Placentomal types between adolescent and adult pregnancies on day 65 and 130 of gestation in Thai-native does are shown in Table 5.3.

The placentomal type A was highest in adolescent does on day 65 of gestation ($39.57 \pm 0.56\%$), followed by adult does on day 65 of gestation ($18.13 \pm 0.97\%$), adult does on day 130 of gestation ($6.62 \pm 2.74\%$), and adolescent does on day 130 of gestation ($5.31 \pm 1.56\%$), respectively. Placentomal type B was higher in adolescent does on day 130 of gestation ($46.92 \pm 3.61\%$) and adult on day 65 of gestation ($38.58 \pm 2.97\%$) than adult on day 130 of gestation ($16.37 \pm 4.76\%$). However, there were no differences in placentomal type B between adolescent and adult goats on day 65 of gestation ($34.73 \pm 0.60\%$ and $38.58 \pm 2.97\%$, respectively, $P > 0.05$). Placentomal type C of adolescent does on day 65 of gestation ($21.21 \pm 0.43\%$) was less ($P < 0.05$) than adolescent does on day 130 of gestation ($21.21 \pm 0.43\%$), adult does on day 130 of gestation ($38.73 \pm 5.63\%$) and adult does on day 65 of gestation ($35.32 \pm 1.40\%$), respectively. Placentomal type D of adolescent does on day 65 of gestation (4.49 ± 0.64) was less ($P < 0.05$) than adolescent does on day 130 of gestation ($21.21 \pm 0.43\%$), adult does on day 130 of gestation ($38.73 \pm 5.63\%$) and adult does on day 65 of gestation ($35.32 \pm 1.40\%$), respectively. The proportion of A+B/C+D ratio in adult does on day 130 of gestation was less (0.40 ± 0.15) than adolescent does on day 130 of gestation ($21.21 \pm 0.43\%$), adult does on day 130 of gestation ($38.73 \pm 5.63\%$) and adult does on day 65 of gestation ($35.32 \pm 1.40\%$), respectively.

The results from the current study indicate that placentomal type A+B/C+D ratio differed between adolescent and adult pregnancies, being lower in adolescent pregnancies. Thus, this ratio may be used to interpret the efficiency of placentomal vascularity.

The maternal age can affect the placentomal type A on day 65 of gestation. There were differences between adolescent ($39.57\pm 0.56\%$) and adult groups ($18.13\pm 0.97\%$, $P<0.01$). In contrast, the placentomal type A on day 130 was not different between adolescent ($5.31\pm 1.56\%$) and adult groups ($6.62\pm 2.74\%$, $P>0.05$). The maternal age cannot affect the placentomal type B on day 65 of gestation. There were no differences between adolescent ($34.73\pm 0.60\%$) and adult groups ($38.58\pm 2.97\%$, $P>0.05$). However, the placentomal type B on day 130 of gestation was highly significantly different between adolescent ($46.92\pm 3.61\%$) and adult groups ($16.37\pm 4.76\%$, $P<0.01$). The maternal age can affect the placentomal type D on day 65 and 130 of gestation ($P<0.01$). Percentages of placentomal type D of adolescent does on day 65 and 130 of gestation were $4.49\pm 0.64\%$ and $7.96\pm 1.15\%$, respectively. Percentage of placentomal type D of adult does on day 65 and 130 of gestation were $7.96\pm 1.15\%$ and $38.29\pm 5.62\%$, respectively (Table 5.3).

Table 5.3 Placentomal type percentage between adolescent and adult pregnancies on day 65 and 130 of gestation in Thai-native does

| Placentomal type (%) | Adolescent (n=8) | | Adult (n=8) | |
|----------------------|-------------------|-------------------|----------------------|-------------------|
| | Day 65 (n=2) | Day 130 (n=2) | Day 65 (n=2) | Day 130 (n=2) |
| A | 39.57 ± 0.56^a | 5.31 ± 1.56^c | 18.13 ± 0.97^b | 6.62 ± 2.74^c |
| B | 34.73 ± 0.60^b | 46.92 ± 3.61^a | 38.58 ± 2.97^{ab} | 16.37 ± 4.76^c |
| C | 21.21 ± 0.43^b | 39.78 ± 3.98^a | 35.32 ± 1.40^a | 38.73 ± 5.63^a |
| D | 4.49 ± 0.64^b | 7.98 ± 1.24^b | 7.96 ± 1.15^b | 38.29 ± 5.62^a |
| A+B/C+D | 2.92 ± 0.12^a | 1.19 ± 0.16^b | 1.38 ± 0.18^b | 0.40 ± 0.15^c |

^{a, b, c} Means in the same row with different superscripts differ significantly ($P<0.05$)

The results from the current studies according to Peninga and Longo (1998) and Ward et al. (2006) reported that the distribution of placentome types changes from A to D type with high altitude hypoxia, which suggesting an acclimatization response to optimize transplacental exchange efficiency. Peninga and Longo (1998) demonstrated that the distributions of placentome types A, B, C and D in ovine single pregnancy were 76 ± 4 , 22 ± 3 , 2 ± 1 and 2 ± 1 placentomes.

5) Vascularity measurements

The procedure of casting and treatment of vascular casts was as follows. The placentomal tissue slides were stained with Hematoxylin-Eosin and Periodic acid schiff. Hematoxylin stains are commonly employed for histologic studies, often employed to color the nuclei of cells (and a few other objects, such as keratohyalin granules) blue. In the current study, Hematoxylin was stained for control slide tissue (Figure 5.3 A). Eosin is a fluorescent red dye resulting from the action of bromine on fluorescein. It can be used to stain cytoplasm, collagen and muscle fibers for examination under the microscope. Eosin is an acidic dye and shows up in the basic parts of the cell, i.e. the cytoplasm. PAS is a staining method used to detect glycogen and other polysaccharides in tissues. The reaction of periodic acid oxidizes the diol functional groups in glucose and other sugars, creating aldehydes that react with the Schiff reagent to give a purple-magenta color. A suitable basic stain is often used as a counterstain (Figure 5.3 B).

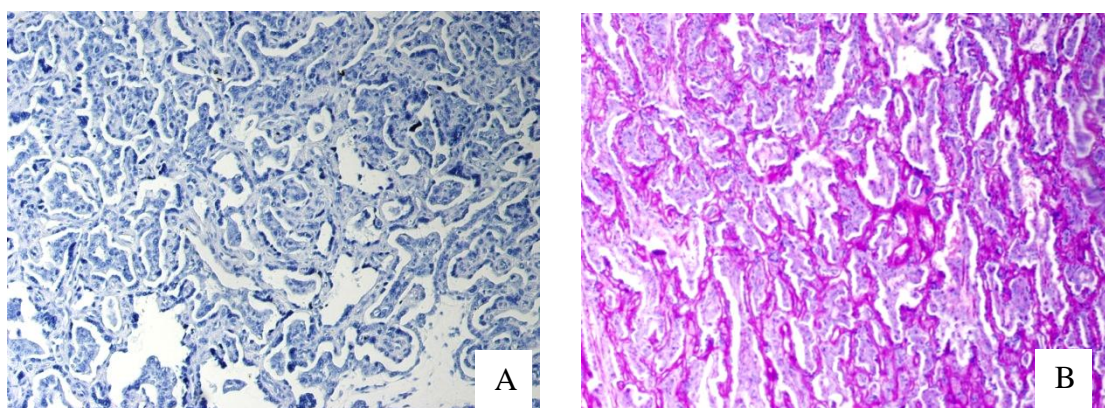


Figure 5.3 Comparative microvasculature photograph in placentomal tissue 20X magnification (A) negative stain (hematoxylin) (B) H&E and PAS stain

Histological pictures of placentomal tissue at 65 and 130 days of gestation from adult and adolescent Thai-native does are illustrated in Figures 5.4 and 5.5. The maternal tissue as a whole showed a tendency to an increase in the number of capillaries while the fetal tissue with the pinkish staining (Periodic Acid Schiff's reagent) represents primarily basement membrane, including those of the microvessels (capillaries, arterioles and venules) and the bluish nuclei represent nuclei counterstain stained with the hematoxylin. The maternal connective tissue (including cells, fiber and blood vessels), syncytium and fetal connective tissue (including cells, fiber and blood vessels), trophoblastic epithelium and binucleated cells are seen. Santos et al. (1998) revealed that the ratio of maternal tissue in the ovine placentome is lower than that in the caprine placentome, nevertheless in the ovine there is also a tendency to an increase in the ratio of maternal tissue at the end of gestation.

1) Capillary area density is the percentage proportion of capillary area per unit tissue. The CAD in CAR tissue was greatest in adolescent pregnancy on day 130 of gestation ($39.97 \pm 1.99\%$), following by adolescent pregnancy on day 65 of gestation ($39.08 \pm 3.64\%$), adult pregnancy on day 65 ($36.97 \pm 3.43\%$), and adult pregnancy on day 130 ($27.74 \pm 3.12\%$), respectively. Mean CAD in CAR tissue between adolescent and adult pregnancies on day 65 of gestation in Thai-native does were 39.08 ± 3.64 and $36.97 \pm 3.43\%$. There were no significant differences between groups ($P > 0.05$). Mean CADs in COT tissue between adolescent and adult pregnancies on day 65 of gestation in Thai-native does were 34.79 ± 1.41 and $43.77 \pm 3.32\%$. Mean CADs in COT tissue in adult does were greater than in adolescent does ($P < 0.05$; Table 5.4). The average CAD in CAR tissue between adolescent and adult pregnancies on day 130 of gestation in Thai-native does was 39.97 ± 1.99 and $35.97 \pm 3.45\%$. There were no significant differences between groups ($P > 0.05$). CADs in adult pregnancy from this study were similar to Redmer et al. (2009), illustrating that as gestation advances, maternal caruncular cellular proliferation declines. However, the proliferative growth in maternal caruncle was not significantly altered by diet.

The results from the present study, show that CAD in COT tissue was highest in adult pregnancy on day 65 of gestation ($43.77 \pm 3.32\%$), following by adolescent pregnancy on day 65 of gestation ($34.79 \pm 1.41\%$), adult pregnancy on day 130 ($33.60 \pm 3.35\%$), and adolescent pregnancy on day 130 ($28.69 \pm 2.70\%$), in sequence.

The average CADs in COT tissue between adolescent and adult pregnancies on day 65 of gestation in Thai-native does was 34.79 ± 1.41 and $43.77 \pm 3.32\%$, respectively. The average CADs in COT tissue between adolescent and adult pregnancies on day 130 of gestation in Thai-native does was 28.69 ± 2.70 and $40.46 \pm 2.90\%$, respectively. Mean CADs in COT tissue in adult does was greater than adolescent does ($P < 0.05$). Redmer et al. (2009) reported that in fetal cotyledon, CAD and CND increased as gestation advanced, whereas APC declined. Wallace et al. (2010) reported that CAD was reduced in the fetal cotyledon from adolescent ewes which were offered high dietary intake (H-group) compared with fetuses from adolescent ewes which were offered an optimal control dietary intake (C-group) on day 50 of gestation.

Figure 5.4 A (adolescent) and B (adult) represent histological micrographs of a caruncular tissue from a pregnant doe at 65 days of gestation. The depth of the crypts increases in correspondence with increased branching of fetal villi. Figure 5.4 C and D represent histological micrographs of caruncular tissue from adolescent and adult pregnant does at 130 days of gestation. The capillary area in a cross section of an adult pregnancy was greater on day 65 of gestation than day 130 of gestation according to Redmer and Reynolds (2009). Binucleated trophoblast giant cells appear in the cotyledonary tissue.

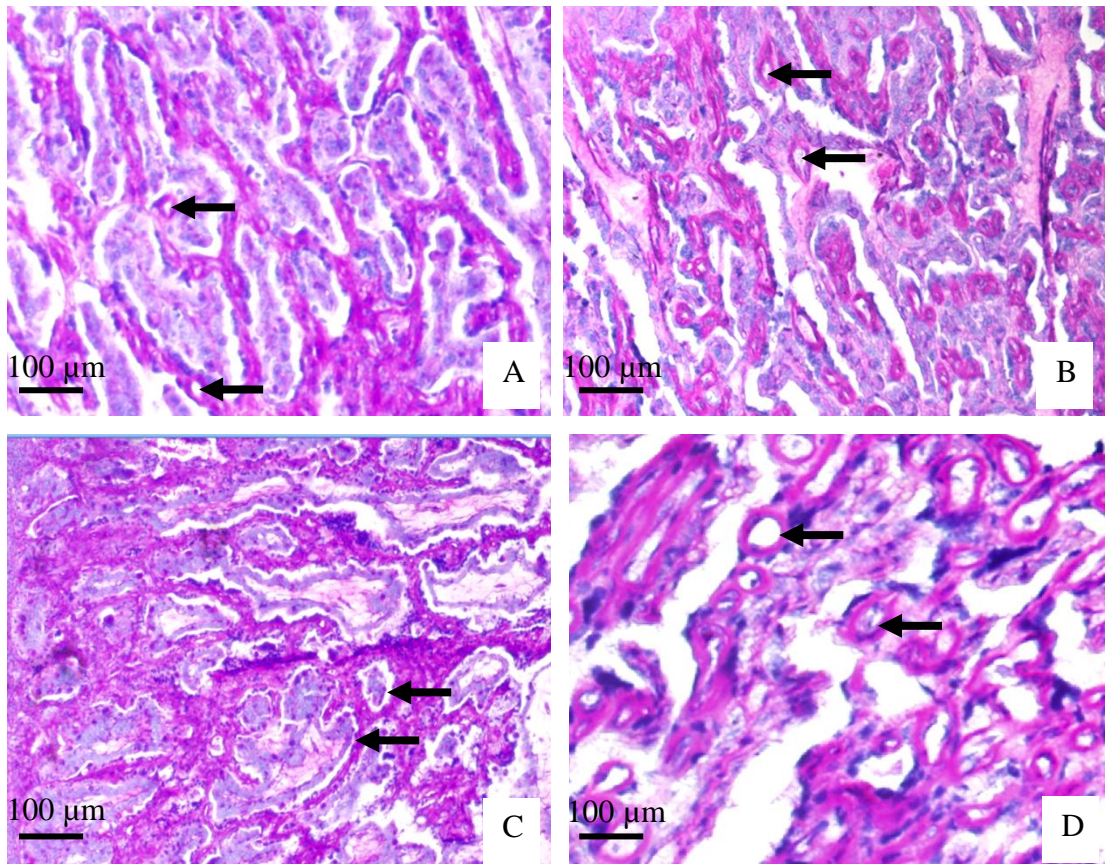


Figure 5.4 Vasculature measurement of caruncular tissue with H&E and PAS staining; (A) in a section of adolescent day 65, (B) in a section of adult day 65, (C) in a section of adolescent day 130, (D) in a section of adult day 130. Size bars indicate 100 μm ; arrows indicate stained capillaries in caruncular tissue. 200X magnification

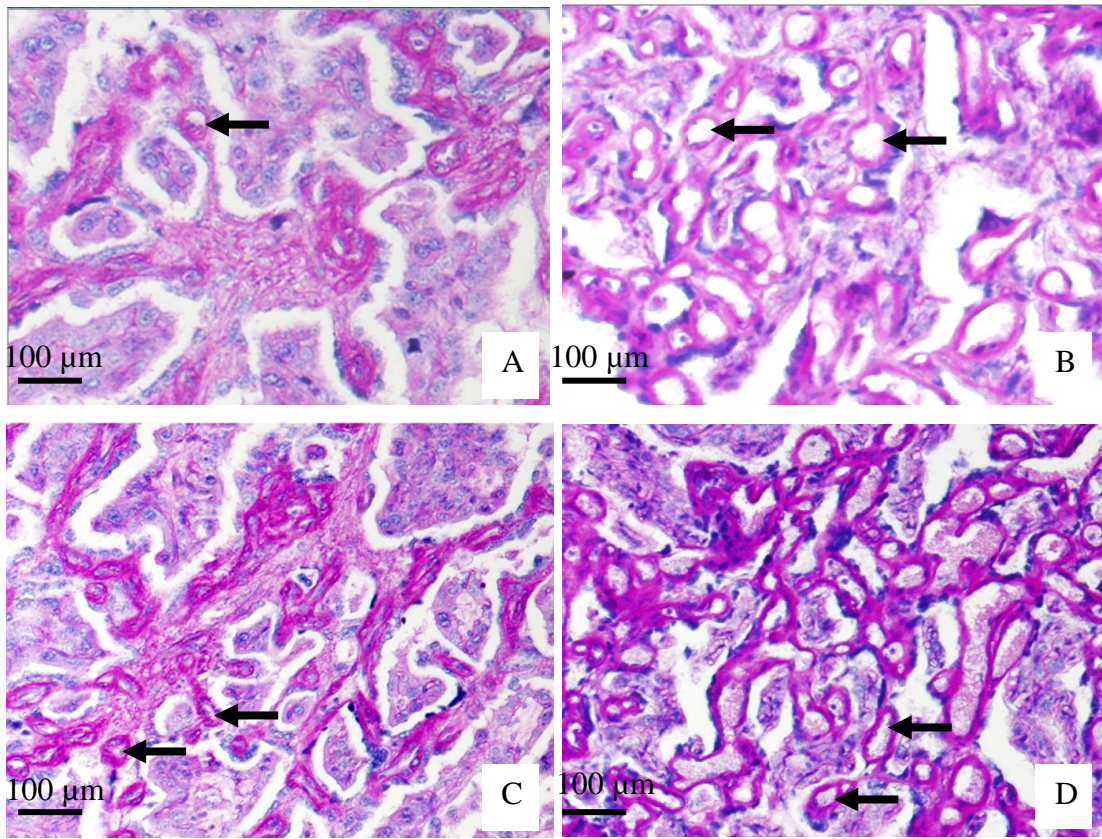


Figure 5.5 Vascularity measurement of cotyledonary tissue with H&E and PAS staining; (A) in a section of adolescent day 65, (B) in a section of adult day 65, (C) in a section of adolescent day 130, (D) in a section of adult day 130. Size bars indicate 100 µm; arrows indicate stained capillaries in cotyledonary tissue. 200X magnification

2) Capillary number density is the proportion of capillary number per unit of tissue. The results from the present study show that mean CNDs in CAR tissue between adolescent and adult pregnancies on day 65 of gestation in Thai-native does were 4.52 ± 0.47 and 4.25 ± 0.69 ($\times 10^{-5}$) no./ μm^2 . There was no significant difference between groups ($P > 0.05$, Figure 5.7). In addition, mean CNDs in COT tissue between adolescent and adult pregnancies on day 65 of gestation in Thai-native does were 6.12 ± 0.55 and 4.93 ± 0.70 ($\times 10^{-5}$) no./ μm^2 ($P > 0.05$). Mean CNDs in total (CAR and COT) tissue between adolescent and adult pregnancies on day 65 of gestation in Thai-native does were 7.16 ± 0.81 and 4.59 ± 0.48 ($\times 10^{-5}$) no./ μm^2 ($P > 0.05$).

Mean CNDs in CAR tissue between adolescent and adult pregnancies on day 130 of gestation in Thai-native does were 9.80 ± 0.77 and 13.56 ± 1.88 ($\times 10^{-5}$) no./ μm^2 ($P > 0.05$). This result is related to the mean CND in COT tissue between adolescent and adult pregnancies on day 130 of gestation in Thai-native does which were 15.71 ± 1.60 and 12.71 ± 1.96 ($\times 10^{-5}$) no./ μm^2 ($P > 0.05$). Furthermore, mean CNDs in total (CAR and COT) tissue between adolescent and adult pregnancies on day 130 of gestation in Thai-native does were 12.75 ± 1.14 and 13.14 ± 1.32 ($\times 10^{-5}$) no./ μm^2 ($P > 0.05$). The study of Borowicz et al. (2007) reported that the CAR and COT portion of the sheep placenta CND increased exponentially. Furthermore, the CAD for CAR and COT increased exponentially from day 50 until day 140 of pregnancy according to Redmer et al. (2004; 2009) revealing that the relative rate of fetal placental (COT) vascular growth exceeds that of the maternal placenta (CAR), especially during the last half of pregnancy. However, Borowicz et al. (2007) indicated that the proportional rate of increase was greater in COT than CAR, Mean CNDs of both adolescent and adult Thai-native does were not significantly different in CAR and COT tissue (Figure 5.8). Redmer et al. (2009) reported that CND in fetal cotyledon increased as gestation advanced.

Figure 5.5 A and B show histological micrographs of CND in cotyledonary tissue from a pregnant doe at 65 days of gestation. Figures 5.5 C and D show histological micrographs of CND in cotyledonary tissue from a adolescent and adult pregnant does at 65 days of gestation. The binucleated cell development is evident in the cotyledonary tissue and there is increase in size.

(3) Area per capillary is the proportion of capillary area in the unit area divided by capillary number. The APC in CAR tissue is shown in Table 5.4, and there was no significant difference in APC between adolescent and adult pregnancies. Mean APCs in CAR tissue between adolescent and adult pregnancies on day 65 of gestation in Thai-native does was $8,844.31 \pm 579.86$ and $9,786.88 \pm 1315.19 \mu\text{m}^2/\text{no}$ ($P > 0.05$). Mean CADs in COT tissue between adolescent and adult pregnancies on day 65 of gestation in Thai-native does was $5,891.46 \pm 405.42$ and $10,023.77 \pm 1,519.77 \mu\text{m}^2/\text{no}$. Mean APC in COT tissue in adult does was greater than adolescent does ($P < 0.05$). The average APCs in CAR tissue between adolescent and adult pregnancies on day 130 of gestation in Thai-native does was $6,216.98 \pm 781.79$ and $4,323.32 \pm 486.43 \mu\text{m}^2/\text{no}$ ($P > 0.05$). The average APCs in total placentomal tissue between adolescent and adult pregnancies on day 65 of gestation were $7,367.88 \pm 508.66$ and $9,905.33 \pm 936.93 \mu\text{m}^2/\text{no}$ ($P > 0.05$). The average APCs in total placentomal tissue between adult does ($4,073.51 \pm 682.34 \mu\text{m}^2/\text{no}$) on day 130 was greater ($P < 0.05$) than adolescent does ($3,200.32 \pm 425.42 \mu\text{m}^2/\text{no}$).

However, APC in COT tissue decreased throughout gestation both in adolescent and adult does. This is similar to Borowicz et al. (2007) who demonstrated that APC decreased by 2.2 fold for COT from day 50 to 140 of gestation in ewes.

Table 5.4 Vascularity measurements in CAR, COT and total tissues between adolescent and adult pregnancy on days 65 and 130 of gestation in Thai-native does

| Vascularity measurement | Adolescent pregnancy (n=4) | | Adult pregnancy (n=4) | |
|---|------------------------------|------------------------------|--------------------------------|------------------------------|
| | Day 65 (n=2) | Day 130 (n=2) | Day 65 (n=2) | Day 130 (n=2) |
| CAR tissue | | | | |
| CAD (%) | 39.08±3.64 ^a | 39.97±1.99 ^a | 36.97±3.45 ^a | 27.74±3.12 ^b |
| APC (µm ² /no.) | 8,844.31±579.86 ^a | 4,323.32±486.43 ^b | 9,786.88±1315.19 ^a | 6,216.98±781.79 ^b |
| COT tissue | | | | |
| CAD (%) | 34.79±1.41 ^b | 28.69±2.70 ^b | 43.77±3.32 ^a | 33.60±3.35 ^b |
| APC (µm ² /no.) | 5,891.46±405.42 ^b | 2,077.31±436.16 ^c | 10,023.77±1519.77 ^a | 1,930.04±261.91 ^c |
| Placentomal tissue (CAR and COT) | | | | |
| CAD (%) | 36.94±1.96 ^{ab} | 34.33±2.18 ^{ab} | 40.37±2.47 ^a | 30.67±2.34 ^b |
| APC (µm ² /no.) | 7,367.88±508.66 ^b | 3,200.32±425.42 ^c | 9,905.33±936.93 ^a | 4,073.51±682.34 ^c |

^{a, b, c} Means in the same row with different superscripts differ significantly (P<0.05)

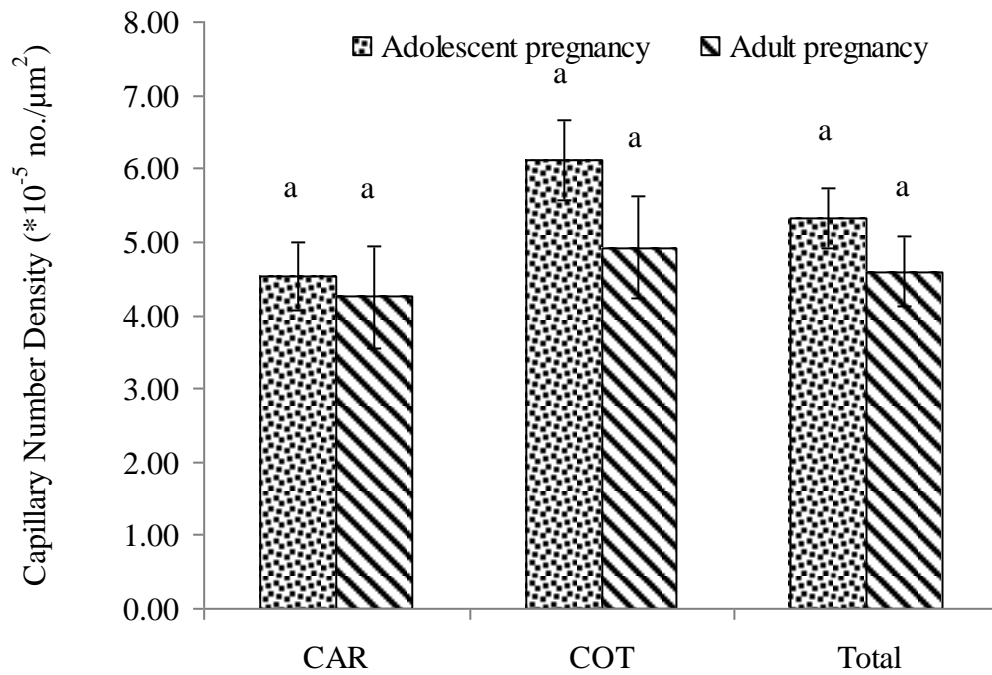


Figure 5.6 Comparison of CND in CAR, COT and total tissue between adolescent and adult pregnancies on day 65 of gestation in Thai-native does

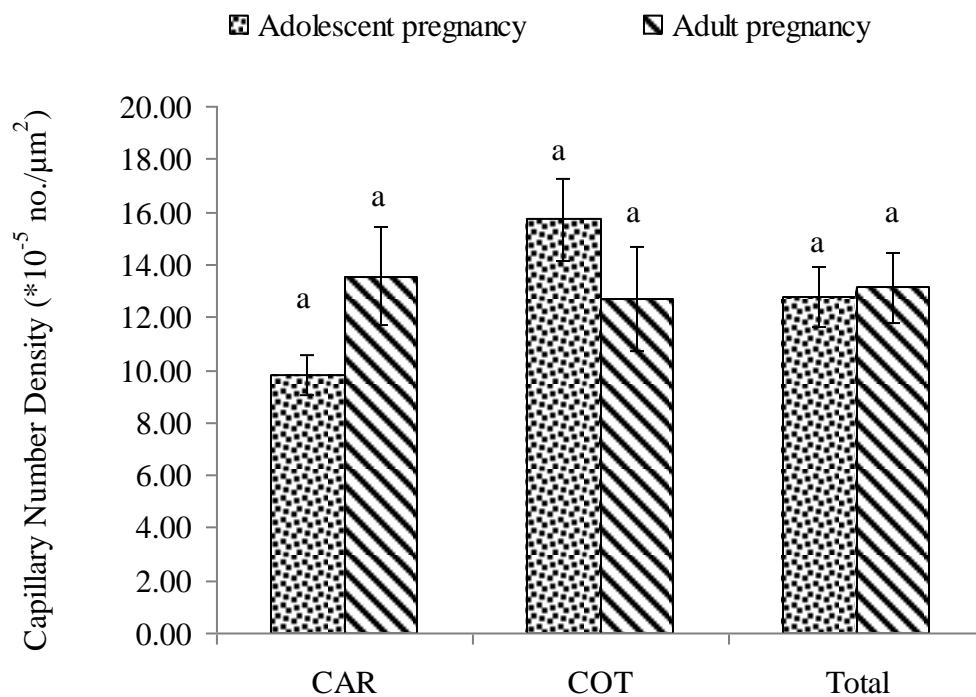


Figure 5.7 Comparison of CND in CAR, COT and total tissue between adolescent and adult pregnancies on day 130 of gestation in Thai-native does

The effect of number of fetuses on vascularity measurement was investigated. The data is shown in Table 5.5. In CAR tissue, CADs was greater in twin pregnancy does (39.97 ± 0.99 no./ μm^2) than in single pregnant does (27.74 ± 3.02 no./ μm^2) but not significantly different from triplets pregnancy does (35.98 ± 3.45 no./ μm^2). CNDs were highly significantly different among triplet (13.56 ± 1.88 no./ μm^2), twin (9.77 ± 0.77 no./ μm^2) and single (4.92 ± 0.77 no./ μm^2) pregnancy does. APCs in single pregnancy does ($6,216.97 \pm 301.96$ μm^2 /no.) were greater than twin ($4,323.32 \pm 144.91$ μm^2 /no.) and triplet ($3,144.70 \pm 78.17$ μm^2 /no.) pregnancy does. In COT tissue, CADs were greatest in triplet pregnancy does ($40.46 \pm 2.90\%$), followed by single pregnancy does ($33.6 \pm 3.35\%$) and twin pregnancy does ($28.69 \pm 2.70\%$), in order. CNDs were not significantly different among groups ($P > 0.05$), by which single, twin and triplet pregnancy does were $18.45 \pm 1.3\%$, 15.71 ± 1.58 and 12.71 ± 1.96 , respectively. APCs in triplet pregnancy doe ($3,857.76 \pm 154.82$ μm^2 /no.) was greater ($P < 0.05$) than in twin ($2,077.31 \pm 132.75$ μm^2 /no.) and triplet ($1,930.04 \pm 100.01$ μm^2 /no.) pregnancies. In total placentomal tissue, average CADs were not significantly different among groups. CADs in single, twin and triplet pregnancy does were $30.67 \pm 2.34\%$, $34.33 \pm 2.18\%$ and $36.94 \pm 1.96\%$, respectively. Average CNDs were not significantly different among groups ($P > 0.05$). CADs in single, twin and triplet pregnancy does was $11.69 \pm 1.89\%$, $12.74 \pm 1.14\%$, and $13.14 \pm 1.32\%$, respectively. Average APCs were not significantly different among groups ($P > 0.05$). APCs in single, twin and triplet pregnancy does were $4,073.51 \pm 682.34$ μm^2 /no., $3,200.32 \pm 425.42$ μm^2 /no., and $3,500.73 \pm 469.55$ μm^2 /no., respectively.

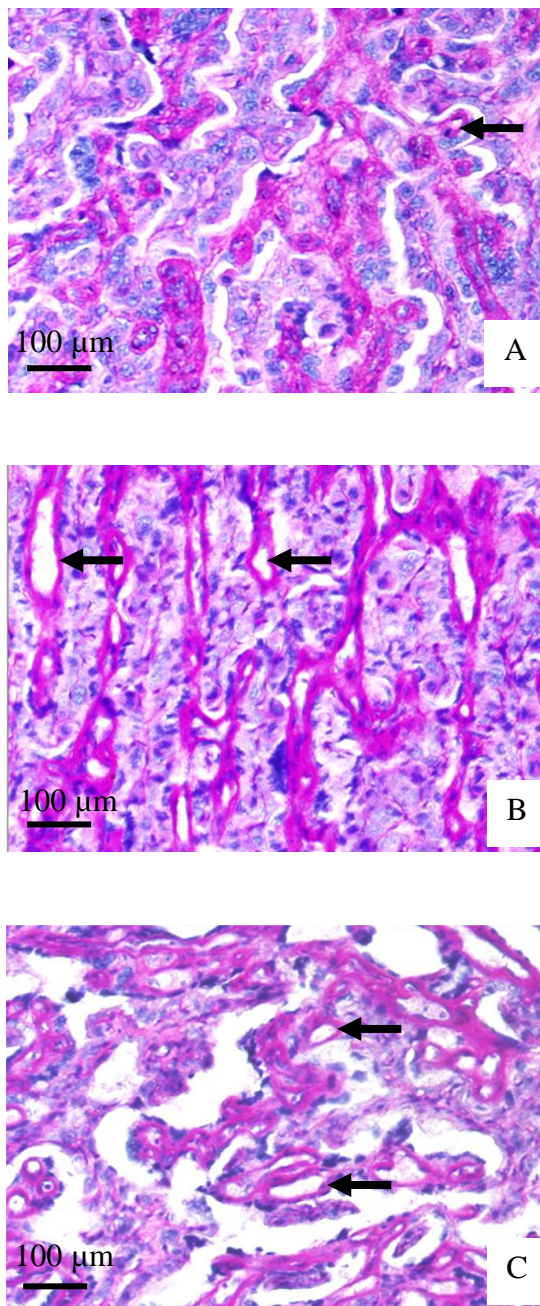


Figure 5.8 Area per capillary in caruncular tissue with H&E and PAS staining; (A) in a section of single pregnancy, (B) in a section of twin pregnancy, (C) in a section of triplet pregnancy. Size bar indicates 100 μm; arrow indicates stained capillaries in caruncular tissue. 200X magnification

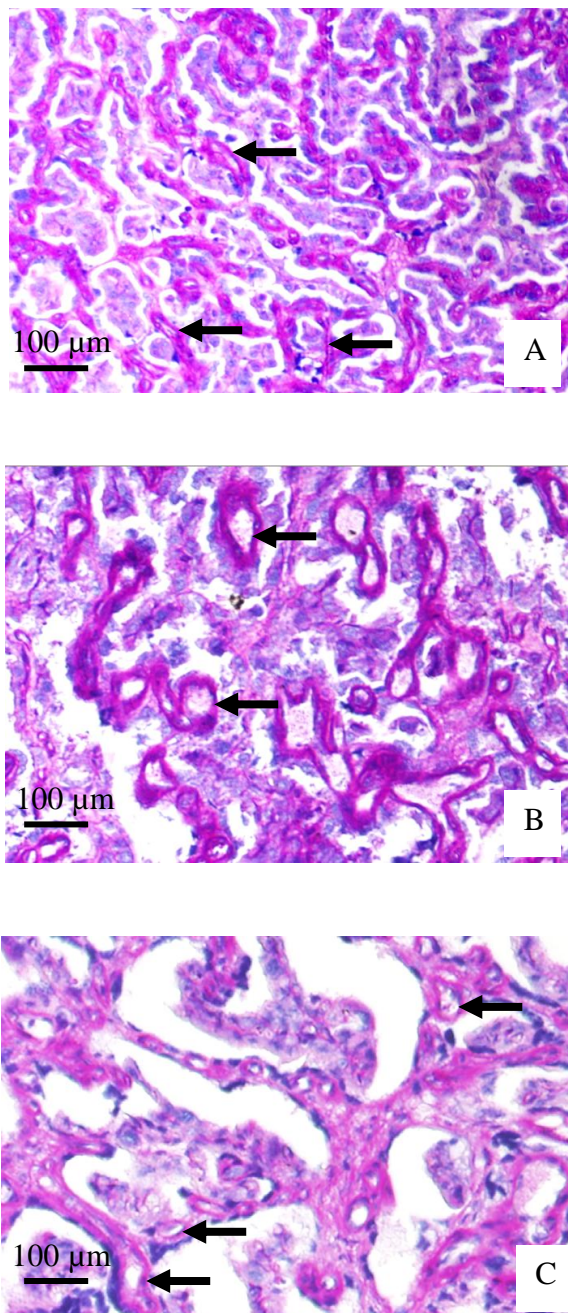


Figure 5.9 Area per capillary in cotyledonary tissue with H&E and PAS staining; (A) in a section of single pregnancy, (B) in a section of twin pregnancy, (C) in a section of triplet pregnancy. Size bar indicates 100 μm; arrow indicates stained capillaries in cotyledonary tissue. 200X magnification

Table 5.5 Vascularity measurements in CAR and COT tissues of single, twin and triplet pregnancies on day 130 of gestation in Thai-native does

| Vascularity measurement | Single (n=3) | Twin (n=4) | Triplet (n=1) | P-value |
|--|------------------------------|------------------------------|------------------------------|---------|
| CAR tissue | | | | |
| CAD (%) | 27.74±3.02 ^b | 39.97±0.99 ^a | 35.976±3.45 ^{ab} | 0.03 |
| CND (x10 ⁻⁵ μm ²) | 4.92±0.77 ^c | 9.77±0.77 ^b | 13.56±1.88 ^a | <0.01 |
| APC(μm ² /no.) | 6,216.97±301.96 ^a | 4,323.32±144.91 ^b | 3,144.70±78.17 ^b | <0.01 |
| COT tissue | | | | |
| CAD (%) | 33.6±3.35 ^{ab} | 28.69±2.70 ^b | 40.46±2.90 ^a | <0.01 |
| CND (x10 ⁻⁵ μm ²) | 18.45±1.3 | 15.71±1.58 | 12.71±1.96 | 0.06 |
| APC (μm ² /no.) | 1,930.04±100.01 ^b | 2,077.31±132.75 ^b | 3,857.76±154.82 ^a | 0.02 |
| Placentomal tissue (CAR and COT) | | | | |
| CAD (%) | 30.67±2.34 | 34.33±2.18 | 36.94±1.96 | 0.06 |
| CND (x10 ⁻⁵ μm ²) | 11.69±1.89 | 12.74±1.14 | 13.14±1.32 | 0.77 |
| APC(μm ² /no.) | 4073.51±682.34 | 3200.32±425.42 | 3500.73±469.55 | 0.51 |

^{a, b} Means in the same row with different superscripts differ significantly (P<0.01)

7) Progesterone concentration

Maternal progesterone concentrations throughout gestation are illustrated in Figure 5.13. Overall, mean serum P4 concentrations were significantly different (P<0.05) between adolescent and adult pregnancies throughout gestational period (days 0, 28, 56, 84, 112, and 140, respectively).

The plasma progesterone concentrations rises significantly from 3.91±0.51 ng/ml on day 40 to 5.9±0.51 ng/ml on day 60 and remains high up to 6 ng/ml until 5 days before kidding. These values in pregnancy are typical of the luteal phase in non-pregnant goats (Llewelyn et al., 1987; 1992). The rise in progesterone level during pregnancy could be due to stimulation of progesterone synthesis by caprine placental lactogen (Currie et al., 1977; 1990) or ovine placental lactogen in sheep which begins to be secreted from day 44 onwards (Linzell and Heap, 1968).

Since placental secretion of progesterone is independent of hypothalamic - pituitary, support, the female does not require LH from anterior pituitary for placental steroidogenesis. The level of progesterone from placental lactogen depends on the number of fetuses present i.e., it increases with the number of fetuses (Hayden et al., 1980); in contrast to this, Humblots et al. (1990) did not find any significant rise in progesterone level according to the number of fetuses. The placental progesterone alone could not maintain pregnancy without the support of corpus luteum in an ovariectomized goat (Sheidrick, et al, 1980).

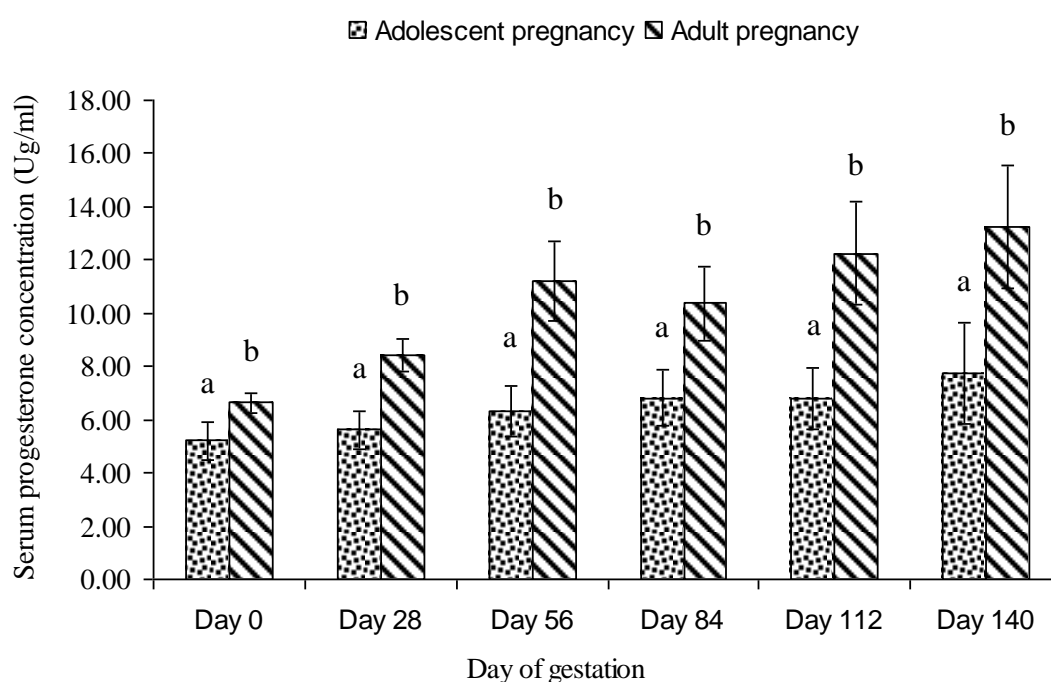


Figure 5.10 Concentration of plasma progesterone in adults and adolescents during days 0-140 of gestation ($P < 0.05$)

8) Cortisol concentration

Serum cortisol concentrations on day 28, 84 and 130 of gestation in adult and adolescent does increased throughout the gestational period. However, there were no significant differences between groups ($P > 0.05$; Figure 5.14). Serum cortisol concentrations on day 0 of gestation (after mating) in adult and adolescent does were 0.38 ± 0.11 and 0.28 ± 0.11 ng/ml, respectively. Serum cortisol concentrations on day 84 of gestation in adult and adolescent does were 0.58 ± 0.14 and 0.55 ± 0.25 ng/ml, respectively. Serum cortisol concentrations on day 84 of gestation in adult and

adolescent does were 1.25 ± 0.32 and 1.25 ± 0.43 ng/ml, respectively. According to Jensen et al. (2007) revealed that in near term ovine fetuses, chronic hypoxia induced VEGF mRNA expression in the placenta. Hyperthermia, which results in an increase in uterine blood flow when expressed per kg of fetus but a decrease in absolute terms, impairs oxygen diffusion in the placenta and results in increased VEGF expression in early gestation, but reduced VEGF expression in late gestation. High cortisol levels in near term resulted in a reduction in uterine blood flow. Thus, relative tissue hypoxia could also have caused VEGF levels to be up regulated in the placenta.

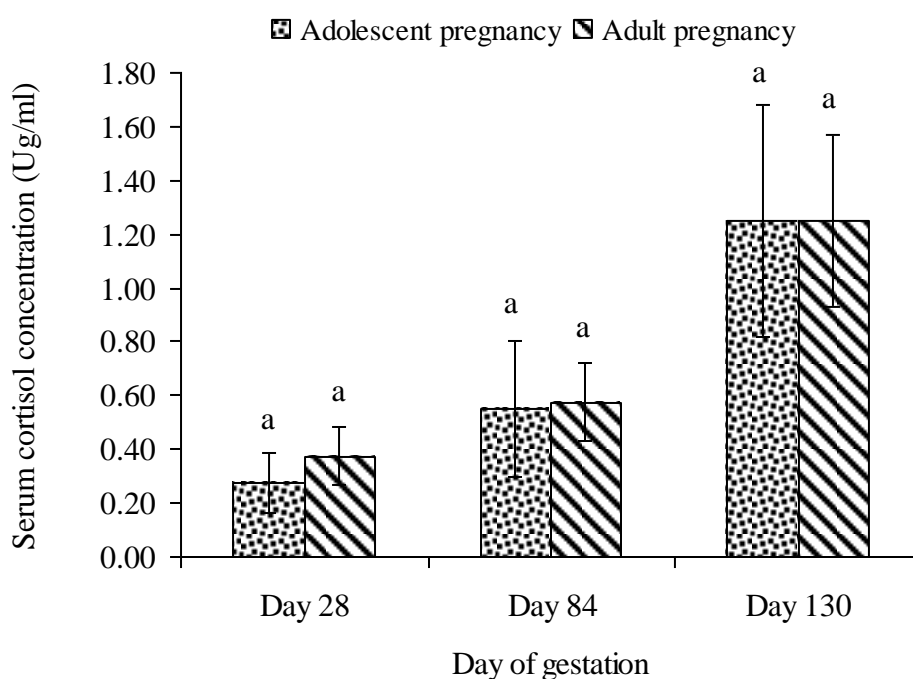


Figure 5.11 Concentration of serum cortisol in adults and adolescents on days 28, 84 and 130 of gestation ($P > 0.05$)

3.2.4 Quantification of VEGF mRNA Expression

1) RNA isolation and real time reverse transcription-PCR (polymerase chain reaction). The total cellular (tc) RNA from the snap-frozen tissue samples were extracted using TRI REAGENT® (Molecular Research Center, Cincinnati, OH). Quality and quantity of tcRNA was reverse-transcribed by using Taqman® Reverse Transcriptase. All cDNAs from the reverse transcription reaction were stored at -20 C

prior to PCR analysis. Expression of VEGF were performed using the Applied Biosystems ABI PRISM® 7300 Sequence Detector (Applied Biosystems, USA) as previously described by Bustin (2000).

Opticon monitor by Chromo4™ Four-Color Real-Time Detector (Bio-Rad, Laboratories Inc. USA.) showed ct cycle and standard curve of VEGF mRNA level in Figure 15.12.

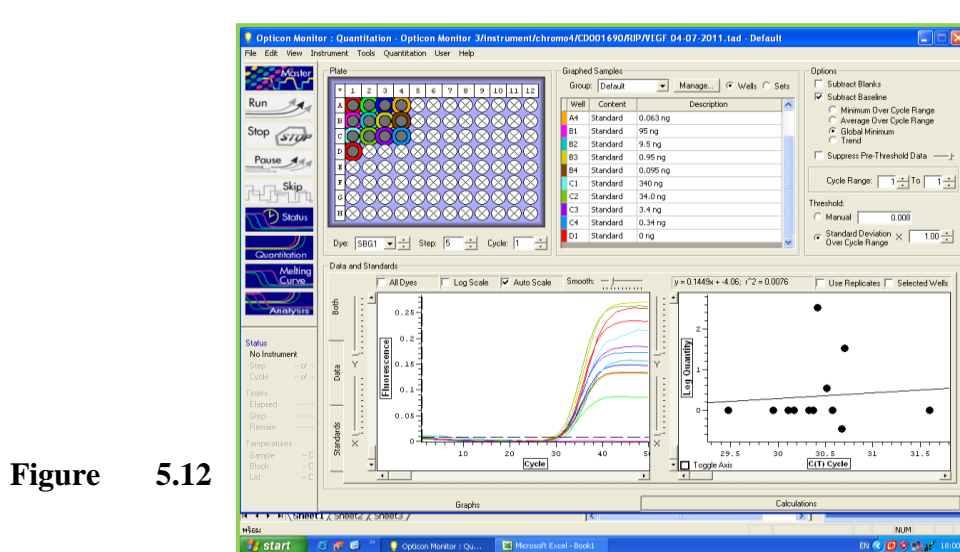


Figure 5.12

Representat

2) The standard curve of mRNA concentration for VEGF

The standard curve of mRNA concentration for VEGF was plotted by using Chromo4™ Four-Color Real-Time Detector (Bio-Rad, Laboratories Inc. USA). Equivalent volumes of stock cDNA from RT reactions into real-time PCR reactions for all candidate endogenous controls were loaded into micro tubes 1.5 ml. cDNAs were diluted with PCR grade water. cDNA concentrations were 281.25, 56.25 and 28.125 ng/μl, respectively. In this study, those standard curves showed a highly linear relationship between ct value and known number template dilution by giving high R^2 values of standard curve as shown in Figure 5.13. PCR grade distilled water was added. cDNA concentrations were diluted to Negative control (distilled water PCR grade)

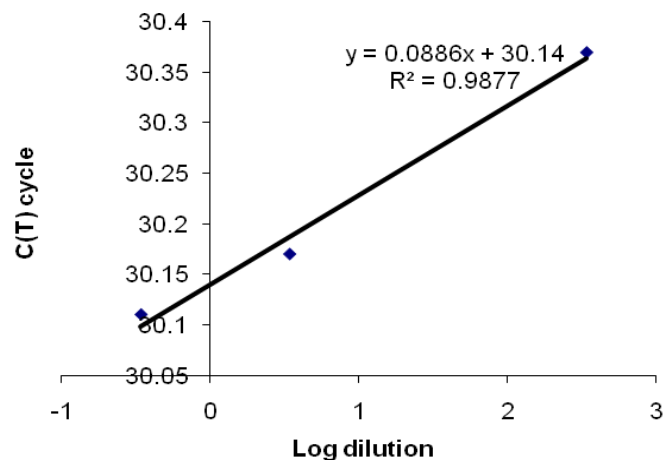


Figure 5.13 The standard curve obtained by plotting the logarithm of mRNA concentration for VEGF165 versus threshold cycle for population quantification by using real-time PCR

However, VEGF is the most important. Five VEGF isoforms are produced by alternative splicing of the same gene, two of them (VEGF121 and VEGF165) are secreted and may act as paracrine or endocrine, whereas the others (VEGF145, VEGF189 and VEGF206) are mostly cell associated and may act as autocrine (Ribeiro et al., 2006). Four VEGF isoforms were identified in the swine corpus luteum. VEGF121 and VEGF165 were the most extensively expressed isoforms, followed by VEGF189 and VEGF145. VEGF189 has previously been described in the ovine corpus luteum (Redmer et al., 1996), follicles during the periovulatory period (Navanukraw, 2003), dominant follicles (Grazul-Bilska et al., 2007), etc.

Cheung et al. (1995) have described that expressions of VEGF were found to increase with gestational age in the sheep placenta. Reynolds et al. (2005) have reported that VEGF expression is strongly correlated with the normal increase in cotyledonary angiogenesis in the placenta in late gestation. Jensen et al. (2007) demonstrated that expression of VEGF mRNA was greater in B type placentomes than A type ($P < 0.05$).

Expressions of VEGF mRNA during gestation in Thai-native does was not different between adolescent and adult does. Jensen et al. (2007) revealed that VEGF was not increased in the low cortisol or low aldosterone animals and there was greater expression of VEGF mRNA in the fetal zone of the placentome than control or low aldosterone animal ($P<0.05$). Furthermore, VEGF protein expression was altered by the differences in maternal steroid treatment. Matsumoto et al. (2002) reported that VEGF mRNA expression is also induced by exposure to low oxygen tension in a variety of pathophysiological circumstances. In the near-term ovine fetus chronic hypoxia induces VEGF mRNA expression in the placenta. However, the cortisol concentrations in this study between adolescent and adult does were not different.

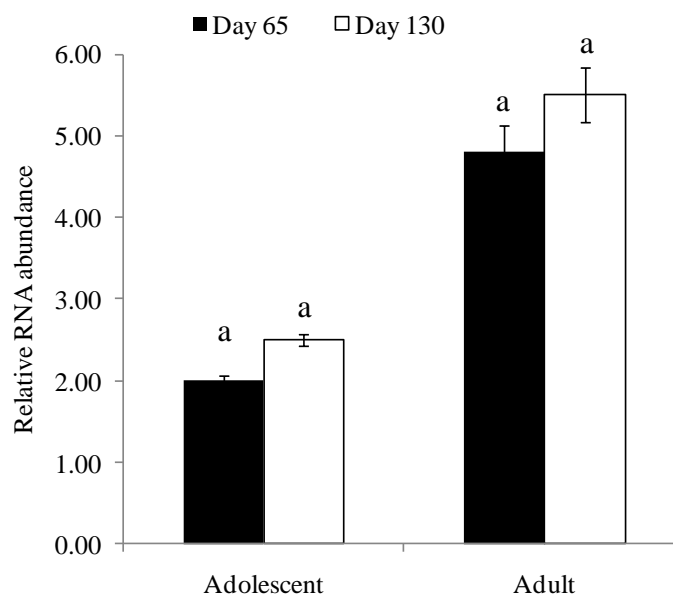


Figure 5.14 The expression of VEGF165 mRNA in CAR tissue between adolescent and adult pregnancies. Different superscripts denote statistically different values ($P<0.05$). Note that only single pregnancy samples were used to quantify mRNA

b

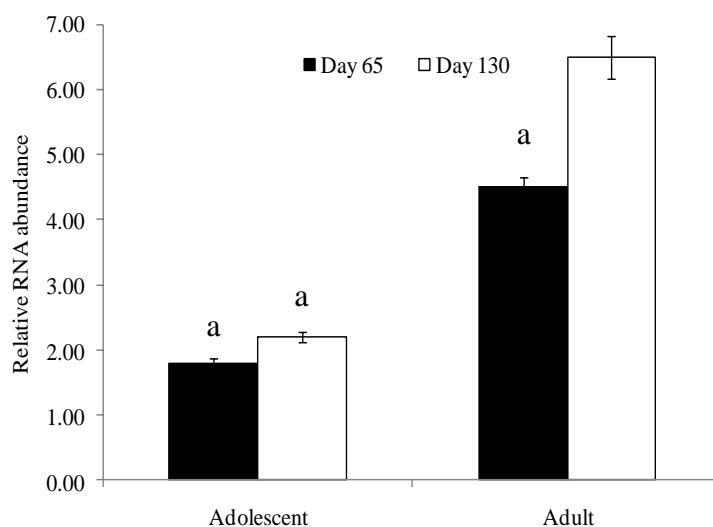


Figure 5.15 The expression of VEGF165 mRNA in cotyledonary tissue between adolescent and adult pregnancies. Different superscripts denote statistically different values ($P < 0.05$). Note that only single pregnancy samples were used to quantify mRNA

5.3 Conclusion

The knowledge from this chapter, indicated that the maternal environment can affect placental development and angiogenesis, which has a large influence during the early implantation period and throughout pregnancy (placental angiogenesis). Maternal age impairs fetal growth.

However, conception and pregnancy rates between adult and adolescent groups were not significantly different (100%) after estrus synchronized with MAP sponge for 14 days. Abortion rate in adolescent pregnancies were higher than in adult pregnancies. This phenomenon was associated with higher concentrations of serum progesterone in adult than in adolescent pregnancies. Furthermore, the placentomal types C and D were more common in adult than adolescent pregnancies.

The placentomal type A+B/C+D ratio can be used to classify the placental function. The caprine placentome can change their shape from A to D type with the

better vascularity in the placenta. However, placentomal type A+B/C+D ratio changed to a lower ratio, thus, which different between adolescent and adult pregnancies. Hence, this ratio may be used to interpret the efficiency of placentomal vasculature. This result indicates that the lower ratio of placentomal type has reverse association with higher concentrations of serum progesterone in adult than in adolescent pregnancies.

The serum cortisol concentrations increases throughout the gestational period but there were no significance different between adolescent and adult pregnancies.

Adult pregnancies are more suitable for implantation than adolescent pregnancies, with the greater capillary area per unit placental tissue CAD in COT tissue ($P < 0.05$). In addition, the CND and progesterone concentrations relate to VEGF mRNA expression of placentomal tissue in Thai-native does.