The Relationship of Progesterone and Estradiol Concentrations During Pregnancy with Lamb Birth Weight In Javanese Thin-Tail Ewes

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ABSTRAK

Tujuan penelitian ini adalah untuk mempelajari hubungan antara konsentrasi progesteron dan estrogen selama kebuntingan dengan bobot lahir pada domba ekor tipis. Tiga puluh sembilan domba bunting digunakan dalam penelitian ini. Domba percobaan disuntik PGF2α dua kali secara intra muskuler dengan interval sebelas hari. Satu-dua hari setelah penyuntikan terakhir domba percobaan dikawinkan secara alami melalui perkawinan kelompok. Sampel darah diambil setiap bulan (0 – 5 bulan) selama periode kebuntingan untuk menentukan konsentrasi progesterone dan estrogen. Bobot lahir anak ditimbang sekitar 12 jam setelah kelahiran. Hasil penelitian menunjukkan bahwa jumlah anak sekelahiran, konsentrasi progesterone dan estradiol serum induk pada bulan kedua kebuntingan secara positif berkorelasi dengan bobot lahir anak. Disimpilkan bahwa semakin tinggi konsentrasi progesterone dan estradiol selama kebuntingan akan semakin tinggi total bobot lahir anak. Disarankan bahwa peningkatan konsentrassi progesterone dan estradiol selama kebuntingan dapat memperbaiki pertumbuhan *prenatal* dan bobot lahir

Key Words: Progesteron, Estradiol, Bobot Lahir, Kebuntingan, Domba

Introduction

Birth weight, which is the resultant of prenatal growth is a significant factor in the survival of lamb during the preweaning period. In sheep, the larger the litter size indicate that the lower lab birth weight often results in a higher preweaning mortality. Prenatal growth is influenced by nutrient supply to developing embryo and foetus that is facilitated by a suitable environment for the uterus and placenta. Maternal hormonal and nutritional status dictates the suitability of environment in the uterus and placenta (Harding and Johnston, 1995; Robinson *et al*., 1995).

Progesterone and estradiol, along with other hormones directly related to pregnancy, are known for their roles in the maintenance of pregnancy through their effects on uterine stromal cell differentiation (Bell, 1984), secretion of uterine milk protein and stimulation of placental growth and hormones secretion during the embryonal stage of pregnancy (Bell, 1984; Wheeler *et al.*, 1987; Mulholland *et al*., 1994). During the fetal stage of pregnancy (2 to 5 months of gestation), progesterone and placental lactogen are reported to influence nutrient flow to the placenta through their effects on the maternal tissue metabolism (Shirling *et al.*, 1981; Sutter-Dub *et al.*, 1981; Fowden, 1995).

Secretion of estradiol and progesterone increase dramatically with the changes in ovarian activity during the estrous cycle and pregnancy. In ovine, secretion of estradiol increases during proestrous along with the maturation of the follicle in the ovary, then decreases significantly during the embryonal stage of pregnancy, and increases precipitously during the fetal stage of pregnancy until parturition (Umo *et al.*, 1976; Pant *et al*., 1977; Manalu *et* *al.*, 1996). Progesterone increases 2 days after ovulation, and iit shows a marked rise from days 5 to a peak between days 7 and 13 (Umo *et al.*, 1976; Pant *et al.*, 1977). It remains almost stable during the first 7 weeks of pregnancy (Manalu *et al.*, 1996).

Previous observation suggest lower peripheral progesterone in overfed animals as a reason for the lower embryonal survival and birth weight (Hard and Anderson, 1979; Parr *et al.*, 1987; Ashworth, 1991). Supplementation of progesterone in the overfed animals during early pregnancy has been demonstrated to restore fetal growth and birth weight similar to those in the control animals (Kendall and Hays, 1960; Parr *et al.*, 1987; Ashworth, 1991). These results indicated that progesterone, and probably other hormones and factors secreted by the ovary, corpus luteum, placenta and uterus, could have significant roles in improving prenatal growth.

During the luteal phase of estrous cycle and the embryonal stage of pregnancy, maternal serum progesterone concentrations are positively correlated with the number of corpora lutea (Quirke *et al.*, 1979). During the fetal stage of pregnancy in goats and sheep (when the placenta is functional), progesterone and estradiol (Manalu *et al.*, 1996), and placenta lactogen (Hayden *et al.*, 1979; Hayden *et al.*, 1980; Butler *et al.*, 1981) also increases with the increased fetal number.

The dramatic change in progesterone and estradiol secretion in sheep and goat could have physiological effects on mammary and prenatal growth. However, there are limited experiments to explore the possible correlation of the endogenous secretion of progesterone during pregnancy with mammary and prenatal growth in polytocous animals. This present study was designed to correlated monthly serum progesterone and estradiol concentrations during pregnancy with mammary growth and lamb birth weight at parturition in Javanese thin-tail ewes.

Methods

Protocol and Experiment Design

This experiment was conducted during the hot (25 to 32°C) and wet (70 to 80% relative humidity) season in it the humid tropics of Indonesia. Thirty nine pregnant Javanese thin-tail ewes were used this experiment with similar body weight (20 to 22 kg) and age (2 to 3 years) at breeding. The experimental ewes were injected twice with PGF2α (Folligon, Intervet, North Holland, Netherland) i.m. at an 11- day interval. One day after the last injection, experimental ewes were mated naturally by group breeding. Blood samples were drawn monthly (months 0 to 5) during gestation period for determine of progesterone and estradiol concentrations. One days after the last injection, experimental ewes were mated naturally by group breeding. At parturition, lamb birth weight was determined around 12 h post partum.

Blood Sampling and Processing

Ten ml of blood samples were drawn with plain vacutainer or sterile syringes from the jugular vein between 09.00 and 10.00 h. The first blood samples were taken one day after the last prostaglandin injection when day estrous (month 0 of pregnancy). Additional blood samples were drawn monthly (30 days) until parturition. Blood samples were allowed to clot in a cool ice box, centrifuged to separate serum (2500 rpm for 30 minutes), which was then frozen for estradiol and progesterone analyses.

Estradiol and Progesterone Analyses

The concentration of serum 17-β estradiol was measured in duplicate by the solid-phase technique radioimmunoassay (Diagnostic Products Corporation, Los

Angeles, CA) using I^{125}-estradiol as a tracer, with a slight modification to accommodate wide ranges of estradiol concentrations in pregnant ovine (Manalu *et al.*, 1996). The estradiol assay used the whole serum withouth prior ether extraction. The radioactivities of I^{125}-estradiol- bound tubes were counted with an automatic gamma counter. The lowest and highest limits of sensitivity of assay were 20 and 150 pg/ml, respectively. Therefore, the concentrations of standard estradiol used to construct the standard curve ranged from 20 to 150 pg/ml. A sample volume of 100 µl serum was used in the assay for samples with estradiol concentrations ranged from 20 to 150 pg/ml. For samples with estradiol concentrations lower than 20 pg/ml, sample volume was increased to 200 to 300 µl to bring the estradiol concentrations to the range of standard used. For samples with estradiol concentrations higher than 150 pg/ml, sample volume was decreased to 50 ul to bring the estradiol concentrations to the range of standard used. All samples estradiol concentrations were within the range of concentrations of standard estradiol used to construct the standard curve. Inter- and intra-assays variation coefficient were 7 and 5 %, respectively. The concentrations of estradiol were parallel in the sample volume of 50, 100, 200, and 300 µl.

The concentration of serum progesterone was measured in duplicate by the solid-phase technique radioimmunoassay (Diagnostic Products Corporation, Los Angeles, CA) using I^{125} progesterone as a tracer, with a slight modification to accommodate wide ranges of progesterone concentrations in pregnant ovine (Manalu *et al.*, 1996). The progesterone assay used the whole serum without prior ether extraction. The radioactivities of I^{125}-progesterone-bound tubes were counted with an automatic gamma counter. The lowest and highest limits of sensitivity of assay were 0.1 and 20 ηg/ml, respectively. Therefore, the concentrations of standard progesterone used to construct the standard curve ranged from 0.1 to 20 ηg/ml. A sample volume of 100 µl serum was used in the assay for samples with progesterone concentrations ranged from 0.1 to 20 ηg/ml. For samples with progesterone concentrations lower than 0.1 ηg/ml, sample volume was increased to 200 µl to bring the progesterone concentrations to the range of standard used. For samples with progesterone concentrations higher than 20 ηg/ml, sample volume was decreased to 50 µl to bring the progesterone concentrations to the range of standard used. All samples progesterone concentrations were within the range of concentrations of standard progesterone used to construct the standard curve. Inter- and intra-assays variation coefficient were 6 and 4 %, respectively. The concentrations of progesterone were parallel in the sample volume of 50, 100, and 200 µl.

Statistic Analyses

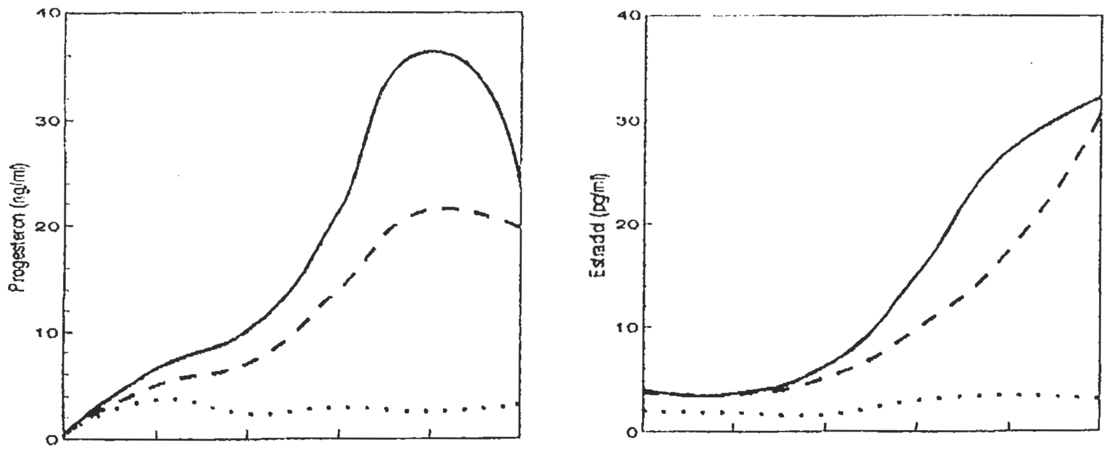
Monthly estradiol and progesterone concentrations during pregnancy were regressed with lamb birth weight using simple regression and correlation analyses (Neter *et al.*, 1985).

Results And Discussion

The profile of maternal serum progesterone and estradiol concentration during pregnancy in the experimental ewe is presented in Figure 1. Progesterone concentration slowly increased during the first 2 months of pregnancy. After that, is increased rapidly and reached peak concentrations around month 3 to 4 of pregnancy and then it decreased near parturition. The concentrations of estradiol were constantly low during the first 2 months of pregnancy. After month 2 of pregnancy, estradiol concentration in the pregnant ewes constantly increased until parturition.

The increased litter size increased the total lamb birth weight curve linearly, with a resultant decreased in average lamb birth weight ($P<0.01$), as is normally observed in prolific ruminants. Total lamb birth weight in the ewes carrying 1, 2, or 3 fetuses were 1.9 ± 0.1; 3.2 ± 0.2, and 4.2 ± 0.4 kg, respectively.

Correlation of monthly maternal serum progesterone and estradiol concentrations during pregnancy with lamb birth weight at parturition are presented in Table 1. Regardless of litter size, the concentrations of maternal serum progesterone during the embryonal stage (months 0 to 2) of pregnancy positively correlated with the total lamb birth weight at parturition. At the other month during this stage of pregnancy, maternal serum progesterone and estradiol concentrations did not significantly correlate with lamb birth weight. During the fetal stage (months 2 to 5) of pregnancy, the concentrations of maternal serum progesterone positively correlated with the total lamb birth weight at parturition. Higher maternal serum progesterone concentrations during the fetal stage of pregnancy associated with the higher total lamb birth weight at parturition. The pattern of relationship between progesterone ant estradiol hormones with lamb birth weight in ewes carrying single and multiple fetuses is presented Figure 2.



Age of Pregnancy (Month)

Figure 1. Monthly serum estradiol concentrations during pregnancy in non pregnant (♦) ewes carrying a single (□) and multiple (♠) fetuses in Javanese thin-tail ewes

Table 1. Coefficients of Correlation between Monthly Maternal Serum Progesterone and Estradiol Concentrations during Pregnancy and Mammary Gland Indices at Parturition in Ewes

Months of Pregnancy	Litter sizes		
	Total	Singel	Multiple
1			
2		80.2	75.0
3		51.0	33.3
4		22.7	19.0
5			

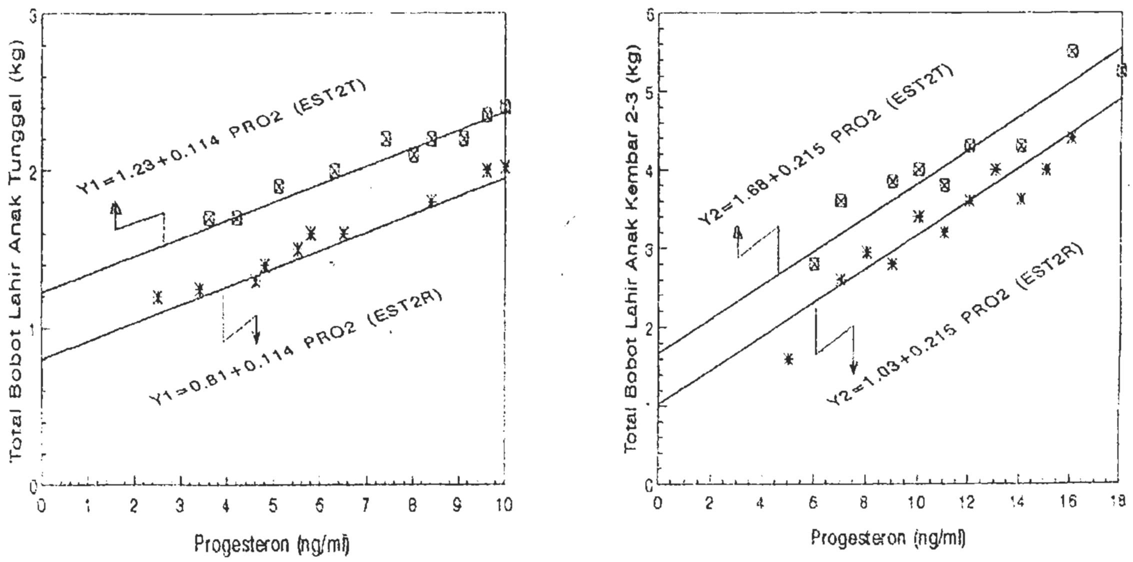


Figure 2. Bentuk Hubungan antara Maternal Serum Progesterone dan Estradiol dengan Bobot Lahir Anak Tunggal dan Kembar

Regardless of litter size, the concentrations of maternal serum estradiol during the embryonal stage (months 0 to 1) of pregnancy positively correlated with the total lamb birth weight at parturition. At the other months during this stage of pregnancy (months 2), maternal serum estradiol concentrations did not significantly correlate ($P>0.05$) with lamb birth weight. During the fetal stage (months 5) of pregnancy, the concentrations of maternal serum estradiol positively correlated with the total lamb birth weight at parturition. In general, higher maternal serum estradiol concentrations during the fetal stage of pregnancy associated with the higher total lamb birth weight at parturition.

The result of this experiment indicated that regardless of litter size, maternal serum progesterone and estradiol concentrations during pregnancy significantly correlated with lamb birth weight. This probably because the total lamb birth weight and maternal serum progesterone and estradiol concentrations during pregnancy increased with the increased litter size (Sumaryadi dan Manalu, 1999). In the ewes carrying multiple fetuses indicated that the higher the serum progesterone and estradiol concentrations the heavier the lamb birth weight.

The effect of progesterone and estradiol on the increased lamb birth weight could have been mediated through their roles in directing gene expression in uterine stromal cells (Muholland *et al.*, 1994) that resulted in stimulation of the uterine and placental growth. Better developed uterine environment could increase nutrients and growth factor secretion and exchanges required to support the developing embryo (Findlay *et al.*, 1981; Bell, 1984; Asworth and Bazer, 1989a,b; Asworth *et al.*, 1989; Asworth., 1991; Asworth, 1992).

Better developed placenta could contribute more to growth of the fetus (Robinson *et al.*, 1995), as nutrients supply in the placenta was not a limiting factor. The effect of progesterone and estradiol concentrations during the fetal stage of pregnancy on lamb birth weight is probably, in part, mediated by the reported effects of progesterone on body energy reserves partitioning to the placenta. Some report indicated that higher progesterone levels could mobilize more fatty acid and glucose from body reserves to maternal circulation (Shirling *et al.*, 1981; Sutter-Dub *et al.*, 1981; Fowden *et al.*, 1995). That in turn could be used by the fetus through the placenta and increased fetal growth (Robinson *et al.*, 1995; Fowden *et al.*, 1995). A better developed placenta produced more placental lactogen and other hormones and Factors (Robinson *et al.*, 1995) that could eventually influence fetal growth through their effects on maternal body energy reserves mobilization (Annison *et al.*, 1984; Bell, 1984; Fowden, 1995) and fetal glycogen synthesis (Hill, 1989; Breier *et al.*, 1994).

Growth and development of the uterine tissue with the overall biochemical change before implantation are initiated by estradiol secreted during preovulation, which are then continued by progesterone (Muholland *et al*, 1994) and probably by other hormones and growth factors secreted by the corpus luteum during the luteal phase of the estrous cycle. Growth and development of the fetus during the fetal stage of pregnancy are affected by the growth and development of placenta (Robinson *et al.*, 1995), nutrients availabilities in the maternal, placental, and the fetal circulations (Harding and Johnston, 1995; Hay, 1995), and the endocrine status of the mother and the fetus (Fowden, 1995).

Conclusion

The result of this experiment indicated that the maternal serum progesterone and estradiol concentrations throughout pregnancy had significantly correlated with lamb birth weight. The result suggested that the increasing maternal serum progesterone and estradiol during pregnancy to improve prenatal growth and lamb birth weight.

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