# Maternal Serum Concentrations of Several Hormones in Does Bearing Different Fetal Number

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**ABSTRACT:** Twelve Etawah-cross (4 cycling-nonpregnant, 5 single and 3 twin) does, with similar body weight and age, were used to study the effect of fetal number on serum concentrations of progesterone, estradiol, triiodothyronine, tetraiodothyronine, and cortisol during the last two months of pregnancy. Blood samples were drawn weekly from jugular vein for hormone analyses using solid-phase technique radioimmunoassay. The results of the experiment showed that average serum progesterone concentrations in the twin-bearing increased ($P<0.01$) by 92.0% as compared to those in the single-bearing does while concentrations of serum estradiol increased ($P<0.01$) by 169%. When compared to cycling nonpregnant does, serum progesterone concentration in the single- and twin-bearing does increased by 1300 and 2,590%, respectively, while estradiol concentration increased by 684 and 2006%, respectively. Average serum triiodothyronine, tetraiodothyronine and cortisol concentrations decreased ($P<0.01$) by 34.9 and 35.3%, 15.7 and 27.6%, and 44.0 and 43.7% in single- and twin-bearing as compared to those in cycling-nonpregnant does. There was no difference in serum concentrations of triiodothyronine, tetraiodothyronine and cortisol between single and twin-bearing does. It was concluded that the increased fetal number from single to twin increased hormonal stimulation (progesterone and estradiol) for mammary gland growth and development during pregnancy. Pregnancy increased utilization of metabolic hormones in the cellular level causing the decreased maternal serum concentrations of triiodothyronine, tetraiodothyronine, and cortisol. However, the increased fetal number in the pregnant does did not seem to affect the utilization of the hormones indicating that hormonal regulations of fetal growth during the second half of pregnancy were independent of maternal endocrine system.

Key Words: Goat, Pregnancy, Estradiol, Progesterone, Thyroid Hormones, Cortisol.

## Introduction

Increased litter size means increased number of corpus luteum and placental mass secreting progesterone and estradiol during gestation period. In sheep and goat fetus grew very fast during the second half of pregnancy. The rapid growth of fetal mass during this period implied a dramatic increased in synthetic activities in the fetus (Rattray et al., 1974).

It was hypothesized that increased fetal number would increase secretion of endogenous progesterone and estradiol to stimulate mammary gland growth and development in preparation of more milk synthesis for the newborn kids. Increased uterine, placental and fetal growth during pregnancy would increase maternal metabolic activities that would be reflected in increased maternal metabolic hormones utilizations. Increased fetal number would increase maternal metabolic hormones utilizations. The objective of this experiment was to study the profile of maternal mammogenic and metabolic hormones concentrations during the fastest growing period of mammary glands and fetus (the second half of gestation period) in different fetal number.

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## Materials and Methods

*Experimental Design and Protocol*

Twelve Etawah-cross does, with similar weight (20 - 22 kg) and age (2 - 3 years), were maintained in the experimental pen with 2 months adaptation to the experimental conditions prior to mating period. During the mating period, the experimental does were mixed with 5 bucks for a month and then the bucks were removed from the flock and the experimental does were maintained in a group of three per pen. Blood sampling were conducted weekly after the breeding period. In the end of experimental period, 8 does gave birth with normal kids (5 singles and 3 twins). Four does were nonpregnant but cycling during the experimental period and were included in data analyses as a control. Experimental does were grouped according to the number of kids born at parturition (0 [cycling nonpregnant as a control], 1 [single] and 2 [twin]), with n = 4, 5, and 3, respectively.

*Blood Sampling and Processing*

Ten milliliters of blood samples were drawn with plain vacutainer or sterile syringes from jugular vein prior to morning feeding at around the same time weekly. The blood samples were allowed to clot in an cool ice box and transported to the laboratory for further separation of serum by centrifugation. The serum samples were then kept frozen for further hormone analyses. Weeks prior to parturition were counted back from the last blood sampling prior to parturition date.

*Hormone Analyses*

*Progesterone.* Concentration of serum progesterone in duplicate was measured by the solid-phase technique radioimmunoassay (Diagnostic Products Corporation, Los Angeles, CA). The radioactivities of progesterone-bound tubes were counted with an automatic gamma counter (Aloka, Model ARC 503, Aloka Co., Ltd., Japan). The concentrations of standard progesterone used to construct a standard curve ranged from 0.1 to 20 ng/ml. All samples progesterone concentrations were within the range of concentrations of the standard progesterone used to construct the standard curve. A sample volume of 100 μl serum was used in the assay.

*Estradiol.* Concentration of serum estradiol in duplicate was measured by the solid-phase technique radioimmunoassay (Diagnostic Products Corporation, Los Angeles, CA). The radioactivities of estradiol-bound tubes were counted with an automatic gamma counter (Aloka, Model ARC 503, Aloka Co., Ltd., Japan). The concentrations of standard estradiol used to construct a standard curve ranged from 20 to 500 pg/ml. All samples estradiol concentrations were within the range of concentrations of the standard estradiol used to construct the standard curve. A sample volume of 100 μl serum was used in the assay.

*Triiodothyronine ($T_3$).* Concentration of serum $T_3$ in duplicate was measured by the radio-immunoassay, solid-phase technique (Diagnostic Products Corporation, Los Angeles, CA). The radio-activities of $T_3$-bound tubes were counted with an automatic gamma counter (Aloka, Model ARC 503, Aloka Co., Ltd., Japan). The concentrations of standard $T_3$ used to construct a standard curve ranged from 20 to 200 ng/dl. All samples $T_3$ concentrations were within the range of concentrations of the standard $T_3$ used to construct the standard curve. A sample volume of 100 μl serum was used in the assay.

*Tetraiodothyronine ($T_4$).* Concentration of serum $T_4$ in duplicate was measured by the radioimmunoassay, solid-phase technique (Diagnostic Products Corporation, Los Angeles, CA). The radioactivities of $T_4$-bound tubes were counted with an automatic gamma counter (Aloka, Model ARC 503, Aloka Co., Ltd., Japan). The concentrations of standard $T_4$ used to construct a standard curve ranged from 10 to 100 ng/ml. All samples $T_4$ concentrations were within the range of concentrations of the standard $T_4$ used to construct the standard curve. A sample volume of 25 μl serum was used in the assay.

*Cortisol.* Concentration of serum cortisol in duplicate was measured by the radioimmunoassay, solid-phase technique (Diagnostic Products Corporation, Los Angeles, CA). The radioactivities of cortisol-bound tubes were counted with an automatic gamma counter (Aloka, Model ARC 503, Aloka Co., Ltd., Japan). The concentrations of standard cortisol used to construct a standard curve ranged from 10 to 200 ng/ml. All samples cortisol concentrations were within the range of concentrations of the standard cortisol used to construct the standard curve. A sample volume of 50 μl serum was used in the assay. This volume was doubled from the volume recommended by the manufacturer to accommodate the lower cortisol concentrations in ruminant animal. Samples ranging from 50 µl to 200 µl were linear to the standard curve.

***Statistical Analyses***

Data during the seven-week measurement were averaged and analyzed for randomized design and the differences between means were tested using least significant difference (Snedecor and Cohran, 1982).

## Results

Averages of serum progesterone, estradiol, $T_3$, $T_4$, and cortisol concentrations of experimental does are presented in Table 1.

Serum progesterone concentrations dramatically increased ($P$<0.01) in the pregnant (by 1300 and 2590% for single- and twin-bearing does, respectively) as compared to those in cycling-nonpregnant does. The results of the experiment showed that the average of serum progesterone concentrations during the last two months of gestation period in the twin-bearing does increased ($P$<0.01) by 92.4% as compared to those in the single-bearing does.

Serum estradiol concentrations also dramatically increased ($P$<0.01) in the pregnant (by 684 and 2006% in single- and twin-bearing does, respectively) as compared to those in the cycling-nonpregnant does. Concentrations of serum estradiol during the last two months of gestation period in the twin-bearing increased ($P$<0.01) by 169% as compared to those in the single-bearing does.

Serum $T_3$ concentrations decreased ($P$<0.01) in the pregnant (by 34.9 and 35.3% for single- and twin-bearing does) as compared to those in the cycling-nonpregnant does. However, there was no difference in serum T3 concentrations during the last two months of gestation period between single- and twin-bearing does.

Concentrations of serum $T_4$ during the last two months of gestation period decreased ($P$<0.01) in the pregnant (by 15.7 and 27.6% for single- and twin-bearing does, respectively) as compared to those in the cycling-nonpregnant does. However, the increased number of fetus from 1 to 2 did not affect average serum $T_4$ concentrations during the last two months of gestation period.

Concentrations of serum cortisol during the last two months of gestation period decreased ($P$<0.01) in the pregnant (by 44.0 and 43.7% for single- and twin-bearing does, respectively) as compared to those in the cycling-nonpregnant does. However, there was no different in serum cortisol concentrations between single and twin-bearing does during the last two months of gestation period.

Table 1. Concentrations of maternal serum progesterone, estradiol, triiodothyronine (T3), tetraiodothyronine ($T_4$) and cortisol of does with different fetal number.[1]

| Fetal number[2] | | Maternal hormone concentrations | | | | |
|---|---|---|---|---|---|---|
| | | Prog.[3] (ng/ml) | Est.[4] (pg/ml) | $T_3$ (ng/dl) | $T_4$(ng/ml) | Cortisol (ng/ml) |
| 0 | Mean | 0.41[a] | 8.78[a] | 111.62[a] | 54.30[a] | 19.02[a] |
| | SE | 0.33 | 0.45 | 6.11 | 2.71 | 1.86 |
| 1 | Mean | 5.79[b] | 68.84[b] | 72.71[b] | 45.78[b] | 10.65[b] |
| | SE | 0.20 | 13.93 | 5.81 | 1.39 | 1.22 |
| 2 | Mean | 11.11[c] | 184.93[c] | 75.26[b] | 39.31[b] | 10.71[b] |
| | SE | 0.64 | 50.01 | 3.76 | 1.58 | 1.86 |

[1]Presented as means and SE of 4, 5, and 3 does with 0, 1, and 2 fetal number, respectively.
[2]Fetal number 0, 1, or 2 refers to cycling-nonpregnant, single- and twin-bearing does, respectively.
[3]Progesterone.
[4]Estradiol.
[a,b,c]Different superscripts in the same column refer to significant difference between fetal number ($P$<0.01).

## Discussion

The results of this experiment implied a dramatic increase in hormonal stimulation for mammary gland growth and development when fetal number increased from 1 to 2. Cycling-nonpregnant does had a basal flat average of progesterone or estradiol concentrations during the same period of measurements, with slight spikes during the estrous cycles. The existence of corpora lutea and placenta during pregnancy dramatically increased serum progesterone and estradiol concentrations. Ranges of progesterone and estradiol concentrations found in goat at comparable ages of pregnancy (Umo et al., 1976) agreed with those reported in this study.

Since progesterone and estradiol were among hormones that played critical roles in mammary gland growth and development during pregnancy (Knight and Peaker, 1982; Anderson, 1986; Tucker, 1986; Tucker, 1987), the increased fetal number or litter size would increase hormonal stimulation for mammary gland growth and development in preparation of more milk synthesis for the newborn kids.

The increased litter size significantly increased mammogenic hormones (Butler et al., 1981) and mammary gland growth and development (Rattray et al., 1974; Butler et al., 1981) in sheep and in goat (Hayden et al., 1979). The increased endogenous mammogenic hormones, especially progesterone, estrogen and placental lactogen, in different litter size was associated with the increased milk production in goat (Hayden et al., 1979) and sheep (Butler et al., 1981).

Ranges of tetraiodothyronine, triiodothyronine and cortisol found in this experiment agreed with those reported in ovine (Reap et al., 1978; Linder, 1959; Linder, 1967). During pregnancy, synthetic activities of does increased due to the rapid growth of uterus, placenta and fetus (Rattray et al., 1974). Increased fetal number also significantly increased protein, fat and energy accretion in the fetal mass (Rattray et al., 1974). $T_3$, $T_4$ and cortisol are hormones involved in growth and synthetic activities, such as protein synthesis (MacDonald, 1980). The increased growth of uterus and placenta (as parts of maternal tissue) during pregnancy involved higher maternal metabolic synthetic activities, requiring more metabolic hormones utilizations. Increased utilization of maternal metabolic hormones ultimately decreased their circulating concentrations in the maternal serum. Increased milk production, as a reflection of higher maternal synthetic activities, caused lower circulating concentrations of thyroid hormones (Vanjonack and Johnson, 1974; Johnson et al., 1991) and cortisol (Johnson et al., 1991) in high-yielding lactating cows. Therefore any increase in metabolic synthesis of maternal tissues or organs is under the control of maternal endocrine system, thereby affecting the circulating concentrations of maternal hormones.

However, the increased fetal energy accretion during gestation period due to the increased fetal number was not reflected in the changes of maternal serum tetraiodothyronine, triiodothyronine and cortisol concentrations. Fetuses are not parts of maternal tissues or organs, and, therefore metabolically independent of the mother in terms of their hormonal regulation of metabolism. However, in term of substrate inflow and metabolic waste disposal, fetuses share the maternal circulation through placenta. It was concluded that the hormonal control of fetal metabolism was independent of endocrine system of the mother during late pregnancy. This conclusion was corroborated by studies reporting that the thyroid and the adrenal cortex of fetus had been functionally active as glands secreting thyroid homones and cortisol prior to birth or when organogenesis had been completed (Nathanielsz, 1975; Nathanielsz et al., 1977).

## Conclusion

The results of this experiment indicated that maternal serum progesterone and estradiol concentrations increased with the increased fetal number from 1 to 2. Maternal serum progesterone and estradiol could be used as strong indices of selection for higher litter size in goat. The increased maternal serum concentrations of progesterone and estradiol suggested an increased hormonal stimulation for mammary gland growth and development during the second half of pregnancy with the increased fetal number from single to twin. Progesterone and estradiol concentrations during the second half of pregnancy could be used as strong parameters to predict whether the does will give birth single or twin kids. Pregnancy decreased maternal serum concentrations of $T_3$, $T_4$, and cortisol. On the other hand, fetal number did not affect the concentrations of maternal metabolic hormones during the second half of pregnancy.

Fetal metabolism during late pregnancy was independent of maternal hormonal regulation.

## Suggestions

Correlation between the increased maternal serum progesterone or estradiol concentrations with the mammary gland growth and development as well as milk production merits further studies for more understanding on hormones controlling mammary gland growth and development. Furthermore, profiles of metabolic hormone in relation to fetal number need to be extended during the whole period of pregnancy.

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