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Investigation of Relationship Between Serum Progesterone, Vitamin A, ß-Carotene and Magnesium Levels During Pregnancy and at Postpartum Period

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In this study, the levels of serum progesterone, vitamin A, ß-carotene and magnesium (Mg) in 12 cows during pregnancy period and after delivery were determined and their interrelationships were evaluated.

Serum vitamin A and ß-carotene, and progesterone levels were assessed with spectrophotometer device, Atomic absorption spectrophotometer and enzyme immunoassay method, respectively.

The progesterone concentration increased slightly (3.02±0.35 ng/ml) at the 2nd month of pregnancy, declined (2.43±0.24-2.44±0.24 ng/ml) between 3-6 months, increased sharply (3.36±0.22 ng/ml) at the 7th month, reached its peak value (3.79±0.35 ng/ml) at 8th month and then declined below 1 ng/ml a day after parturition. The present results revealed that gestation had a significant ($P<0.001$) effect on concentrations of serum progesterone, vitamin A, β-carotene and Mg. A positive correlation was found between serum progesterone and Mg ($P<0.01$), progesterone and vitamin A ($P<0.05$) or progesterone and β-carotene ($P<0.05$) during gestation. Additionally, there was an important correlation between serum vitamin A and ß-carotene during pregnancy ($P<0.01$).

In conclusion, the present findings suggest that serum progesterone, vitamin A, ß-carotene and Mg concentrations of the cows similarly fed, could alter depending on gestation period and parturition.

Key Words: *Cows, Pregnancy, Progesterone, Vitamin A, ß-Carotene.*

Gebelik Boyunca ve Doğumda Serum Progesteron, Vitamin A, ß-Karoten ve Magnesyum Arasındaki İlişkinin Araştırılması

Bu çalışmada, toplam 12 inekte gebelik ayları ve doğum sonrası serum progesteron, vitamin A, ß-karoten ve magnesyum seviyeleri ve bu parametreler arasındaki ilişki araştırıldı.

Kan serumu (Mg) düzeyleri Atomik absorbsiyon spektrofotometresi, vitamin A ve ß-karoten apektrofotometre ve progesteron değerleri enzim immuno assay metoduna göre ölçüldü.

Progesteron düzeyinde gebeliğin 2. ayında (3.02±0.35 ng/ml) hafif bir artış, 3. aydan 6. aya kadar (2.43±0.24-2.44±0.24 ng/ml) azalma, 7. ayda (3.36±0.22 ng/ml) keskin bir artış, 8. ayda (3.79±0.35 ng/ml) pik düzeye ulaştığı ve doğum sonrasında 1 ng/ml altına düştüğü belirlendi. Serum progesteron vitamin A, β-karoten ve Mg düzeyleri gebelik dönemine göre değiştiği ($P<0,001$) tespit edildi. Gebelik boyunca progesteron-Mg ($P<0,01$), progesteron-vitamin A ($P<0,05$), progesteron-β-karoten ($P<0,05$) arasında pozitif korelasyonun olduğu belirlendi. Ayrıca vitamin A-β-karoten arasında da korelasyonun ($P<0,01$) olduğu görüldü.

Sonuç olarak, aynı şartlar altında beslenen ineklerde serum progesteron, vitamin A, β-karoten ve Mg düzeylerinin gebelik dönemi ve doğuma bağlı olarak değiştiği tespit edildi.

Anahtar Kelimeler: *Gebelik, progesterone, Vitamin A, β-karoten, Mg, inek.*

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Introduction

Vitamin A is necessary for animals to have normal growth, reproduction and health condition. Under normal circumstances the cattle do not ingest natural vitamin A but enable to produce it from provitamins such as ß-carotene contained mostly in green feed. ß-carotene, a vitamin A precursor, converted mostly by the mucosa of the small intestine and appears to be the most efficient provitamin (1). been reported (ref) that the cattle has a specific requirement to ß-carotene for luteal development, progesterone (P4) production and fertility. It has Periparturient cows undergo intense mammary growth and marked production of colostrum rich in vitamin A, ß-carotene during gestation, its circulatory levels decreases at the parturition, which is reported to predispose such animals to severe health problems including mastitis, retained placenta and metritis (2, 3).

Progesterone is required for the maintenance of pregnancy in animals. It is a hormone with different actions dependent on the periods in the oestrous cycle and regulates maturation of the oocytes, ovulation, myometrial quiescence, mammary gland growth and endometrial enzymes. Progesterone follows a regular pattern during pregnancy and declines in the prepartum period and at parturition in cattle. The natural progesterone levels in plasma show a large variation, depending on the species, sex, age and physiological status (4, 5, 6).

The objective of the study was investigate the effects of gestation and parturition on progesterone, vitamin A, ß-carotene and Mg concentrations and relation among these parameters in cow blood serum housed and feed under the same conditions.

Material and Methods

Animals: Totally 12 cows (average body weight 350-400 kg) between 3-8 years (5 Holstein, 5 Swiss-Brown and 2 Simmental) were used as material. The materials were chosen among the animals belonged to Fırat University, Faculty of Veterinary, Research and Implementation Farm.

All animals were under the same care and feeding conditions. All animals were fed with a ration consisting following ingredients. Dry matter 93.75%, ash 5.09%, crude fiber 9.75%; crude protein 15.18%; ether exract 5.80%, organic matter 88.66%, barley 70.50%, sunflowerseed meal 17.50%, soybean 7.50%, limestone 3.00%, DCP (dicalsium fosfat) 0.50%, salt 0.50%, vitamin 0.25%, trace mineral 0.25%. Composition of forage feed as follows. Dry matter 95.20 %, ash 9.47%, crude fiber 35.00 %, crude protein 3.30%, ether exract 3.20%, organic matter 85.73%. In the morning and evening animals were given the ration of which details were given above.

The estrus of the animals was synchronized with PGF2α. The cows at the period of estrus were inseminated naturally. Estrus and insemination dates were recorded. The pregnancy condition of the animals was determined by B mode ultrasonography (100 Falco, Pie Medical Application Manual, Equipment B.V., Maastricht, Netherland) between the 30th and 35th days after insemination.

Serum and assay procedures: Following mating on the 30th day, a blood sample of 10 ml was taken from each animal by using jugular vein sterile blood taking tubes. Then the blood samples were taken monthly during the pregnancy and within 24 hours following the delivery. The blood samples were kept at room temperature for 2 hours in order to obtain the sera. The sera taken into the centrifuge tubes were centrifuged at 3000 rpm for 15 minutes. The sera, which were taken into serum preservation tubes were stored at -20 oC until the analysis was concluded.

Serum samples were analyzed using a double-antibody EIA technique for determination of P4 according to Prakash et al. (7). All assays were carried out in 96 well microtitre plates (Nunc-Immunoplate, Cat. No. 439454, Brand Products, Denmark) and standards, samples and controls were studied in duplicate. The range of standards for P4 was from 0.25 to 16.0 ng/ml . Intensity of colour measured at 450 nm with an 8-channel microtitration plate photometer (Tecan, Spectra III, A 5082, Austria) and results were evaluated using EasyWin Kinetics software supplied by Tecan. The sensitivity of assay was 0.11 ng/ml and intra- and inter assay coefficients of variations were 7 % and 14 %, respectively. Vitamin A and ß- carotene values were determined spectrophotometrically by Suzuki and Katoh's method (8). The levels of Mg were determined by Atomic Absorption Spectrophotometer (Perkin Elmer 370) (9,10).

Data analysis: Results were expressed as mean ±SEM. In respect of statistical calculations, the variations among the pregnancy months were found by implementing the ANOVA and the significance of such variations was also noted due to the Duncan Test. The correlation coefficients in the among parameters were determined by pearson test (11). A level of P<0.05 was considered statistically significant.

Results

In this study, the mean concentrations of serum progesterone, vitamin A, β-carotene and Mg during pregnancy and at parturition, and monthly variations belonging to totally 12 pregnant cows were shown in Table 1. Gestation period had a significant (P<0.001) effect on levels of progesterone (P<0.001), vitamin A (P<0.001), β-carotene (P<0.01) and Mg (P<0.001). Serum vitamin A and β-carotene levels after parturition were lower than prepartum period (Fig.1). Furthermore, it was determined that although the level of serum Mg after parturition was lower than the prepartum; no statistical difference (P>0.05) was observed. The concentrations of progesterone increased slightly at 2 month of pregnancy, and declined from 3 to 6 months, increased sharply at 7 month, peaked at 8 month and then declined below 1 ng/ml a day after parturition.

Serum progesterone concentration was positively (r=0.83, P< 0.01) correlated with Mg concentration during gestation. Also, during the gestation period, plasma progesterone was positively correlated with both vitamin A (r=0.69, P<0.05) and β-carotene (r=0.69, P<0.05). It was noted that there was an important correlation between serum vitamin A and ß-carotene during pregnancy (P<0.01). Length of gestation in cows was determined as mean 282.83±1.15 days. One of the cows had female twins born, and 6 female and 7 male of the calves. Concentrations of serum progesterone during pregnancy in female and male-bearing cows were 2.49±0.28 and 2.53±0.27 ng/ml, and sex of calves had no effect on levels of progesterone.

Table 1. Concentrations of serum progesteron, vitamin A, β-carotene and Mg during gestation period in cows (n=12).

Months of pregnancy	Parameters			
	Progesteron(ng/ml)	A vit. (µg/dl)	β-carotene (µg/dl)	Mg (mg/dl)
1	2.22±0.32 [bcd]	52.97±3.11 [bc]	216.82±12.84 [bc]	2.15±0.014 [a]
2	3.02±0.35 [def]	49.76±3.11 [bc]	199.43±8.24 [abc]	2.86±0.38 [bcd]
3	2.43±0.24 [bcd]	45.60±2.15 [ab]	202.77±10.62 [abc]	2.29±0.17 [ab]
4	2.04±0.24 [b]	46.43±2.52 [abc]	188.21±9.80 [ab]	2.17±0.12 [a]
5	2.14±0.25 [bc]	47.87±1.74 [bc]	198.06±6.73 [abc]	2.42±0.18 [ab]
6	2.44±0.24 [bcd]	50.70±2.10 [bc]	224.26±8.01 [c]	2.60±0.21 [abc]
7	3.36±0.22 [ef]	53.85±2.94 [c]	223.30±11.30 [c]	3.17±0.19 [cd]
8	3.79±0.35 [f]	50.90±2.63 [bc]	216.37±15.19 [bc]	3.30±0.34 [d]
9	2.92±0.28 [cde]	47.97±1.22 [bc]	218.34±5.70 [bc]	2.84±0.25 [bcd]
Parturation	0.61±0.10 [a]	40.22±1.53 [a]	180.44±3.52 [a]	2.22±0.12 [ab]
P	P<0.001	P<0.001	P<0.01	P<0.001

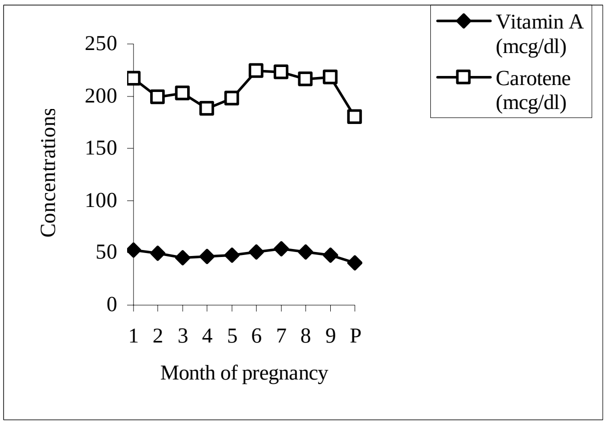

Figure 1. Serum vitamin A and β-carotene changes during pregnancy of cows.

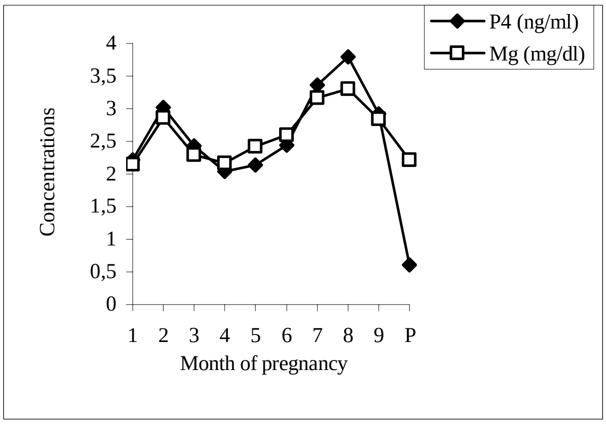

Figure 2. Serum progesterone and Mg changes during pregnancy of cows.

Discussion

Concentrations of vitamin A and β-carotene during pregnancy period ranged from 40.22±1.53 to 53.85±2.94 µg/dl and 180.44±3.52 to 224.26±8.01 µg/dl, respectively. Similar results were reported in pregnant cows by some researchers (2, 12, 13). The evaluation of serum vitamin A level in other month, except the 3rd and 4th months of pregnancy, showed statistically significant decrease (P < 0.001) with the lowest values at the parturition. The β-carotene levels after parturition were lower than the 1st, 6th, 7th, 8th, and 9th months of the pregnancy (P < 0.01). Our findings are comparable to the data obtained at various month of pregnancy and after parturition in pregnant cows (2, 12, 13). Aksakal et al. (14) reported that β-carotene levels were sequentially 177, 190, 168, 263, 125 and 101 µg/dl in the 5, 6, 7, 8 and 9th months of pregnancy, plasma β-carotene levels were determined to decrease continually in the late pregnancy period, and the minimum levels were observed in the delivery period.

During the last weeks of pregnancy and at parturition a decrease in vitamin A and β-carotene levels in blood is very pronounced, probably due to the utilization of vitamin by cows organism in order to ensure an abundant intake of this substance of vital importance to a calf (12). The vitamin A and β-carotene decreases in plasma of prepartum cows are, according to Thompson (15), probably caused by a vitamin A and β-carotene transfer to colostrum and not by hormonal changes as generally stated. It was noted that there was important correlation between serum vitamin A and ß-carotene during pregnancy (r= 0.69, P<0.01). Johnston and Chew (16) also determined that there was a significant correlation between plasma vitamin A and β-carotene levels in the pregnancy and postpartum periods of the cows. They also found a correlation in positive (P<0.05) between serum vitamin A and progesterone; β-caroten and progesterone during pregnancy. It is claimed that there is correlation between serum β-carotene levels and progesterone values, and correlation between serum vitamin A and progesterone levels in cows (17). In another study performed with the follicle fluid of simmental cows (18), correlation between progesterone and vitamin A was noted. Positive correlation between progesterone and vitamin A or β-carotene during gestation indicated that vitamin A and β-carotene together with progesterone may have an effective role in the development of corpus luteum which provides the continuation of pregnancy, and on the initiation of the progesterone synthesis, as the vitamin A and β-carotene levels in corpus luteum in the luteal phase is reported to be higher compared to the other periods of the cycle (19).

The individual results indicated that values of progesterone increased slightly at 2 month of pregnancy, declined from 3 to 6 months, increased sharply at 7 month, peaked at 8 month and then rapidly declined a day after parturition. Eissa et al. (4) concluded that the concentration of progesterone increased slightly during the first 2 months and 4 months of pregnancy. Their values reached peak these levels at 7 month of the pregnancy period, decreased significantly at days 7 prepartum and dropped sharply to below 1 ng/ml after parturition (4, 20). Eissa and El-Belely (21) reported that the plasma concentrations of progesterone increased until the 3rd month of gestation, then decreased significantly at the 4th month. The higher values of progesterone during the first trimester of gestation might be associated with the formation of accessory luteal tissue arising from an ovulation which frequently occurs within the gestation period. The obtained results were in contrast to those previously reported in the pregnant cows (22). They did not find any differences in plasma progesterone concentrations during the months of gestation. The findings are in agreement with that reported by some researchers (2, 20, 21).

The serum progesterone profile paralleled the serum Mg profile throughout gestation and at parturition in the cows (Fig. 2). Therefore, strong positive correlation between serum progesterone and Mg was observed in our study. It was reported that plasma progesterone concentration was positively ($P<0.05$) and negatively ($P<0.05$) correlated with Mg concentration during gestation and lactation respectively. Also, during the dry period, plasma progesterone was positively ($P < 0.05$) correlated with Mg (23). The positive correlation between plasma concentrations of progesterone and Mg during pregnancy may therefore be a result of parallel increases of plasma progesterone to maintain the conceptus and increases of plasma Mg in response to raised metabolic rate to meet the demands of both the cows and the growing foetus.

The mean values of Mg during pregnancy and at parturition ranged from 2.15 to 3.30 mg/dl and they are in coincidence with the data recorded during pregnancy in cows of some researchers (24, 25). Shahzad et al. (26) reported that level of serum Mg after parturition was higher than prepartum period with no observed puerperal disorders. However, gestation period in cows had no determined effect on concentrotions of Mg (27). In this study, reached peak 8 month of pregnancy, values of serum Mg after parturition lower than 8 month of pregnancy were observed. Similar findings were reported by Jacob et al. (24) during gestation period in cattle. These results indicated that Mg levels in cows may be changeable according to gestation period

Lammoglia et al. (28) reported that on 20 to 14 days before calving, progesterone concentrations in cows bearing male calves were greater than those in cows bearing female calves, but on 13 to 7 days before parturition, progesterone concentrations were not significantly different. In contrary, it was observed that the sex of the foetus in sheep did not seem to influence plasma progesterone levels and plasma progesterone concentrations, which do not appear to be a useful tool in predicting the sex of the foetus (29). In this study, concentration of serum progesterone during pregnancy and at parturition in cows carrying male (2.53±0.27 ng/ml) was similar to that in cows carrying famele (2.49±0.28 ng/ml). These findings were in agreement with results reported in sheep by Kalkan et al. (29).

In conclusion, according to this study, it is suggested that gestation period and parturation may influence the concentrations of serum progesterone, vitamin A, ß-carotene and Mg of the animals under same condition. Serum vitamin A, ß-carotene and Mg as well as progesterone are required for the maintenance of pregnancy in cows.

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