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Serum Progesterone, Vitamin A, E, C and β-Carotene Levels in Pregnant and Nonpregnant Cows Post-Mating

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Abstract: The aim of the study was to detect the progesterone, vitamin A, E, C and β-carotene levels in blood serum and the relationship between them in pregnant and nonpregnant cows after insemination. The oestrus of 17 cows kept at the same maintenance and nutrition conditions were synchronized by prostaglandine F2-alpha. The cows that were in heat were naturally inseminated. Blood samples were taken every four days starting from the oestrus day, throughout the whole cycle. Pregnancies were detected by ultrasonography in between days 30-35 after insemination. 12 cows were detected to be pregnant this way, while 5 cows were detected to be not pregnant. Serum progesterone levels were determined by enyzme immunoassay method; and vitamin A, E, C and β-carotene levels were detected by spectrophotometry. While no difference was observed between the sampling days in serum progesterone, vitamin A, C, E and β-carotene levels in bloods collected for 21-24 days after insemination in pregnant and nonpregnant cows ($P>0.05$), vitamin C level at the 16th day after insemination in pregnant cows was detected to be higher ($P<0.05$) compared to the nonpregnant cows. It was observed that the average serum vitamin A, E and β-carotene levels were higher during the cycle in pregnant cows compared to the nonpregnant cows ($P<0.001$) and that there was no difference with regard to vitamin C levels between the groups ($P>0.05$). A negative correlation between serum progesterone and vitamin A and progesterone and vitamin E ($P<0.01$); and a positive correlation between serum vitamin E and A levels ($P<0.01$) were detected during the cycle in pregnant cows. As a conclusion, serum progesterone, vitamin E, A and β-carotene levels were observed to be higher in the pregnant cows following insemination compared to the nonpregnant cows and a positive correlation with pregnancy was detected.

Key words: Vitamin, A, E, C, β-Carotene, Pergnant, Nonpregnant, Post-mating

Introduction

Vitamins, along with carbohydrates, proteins and minerals taken through ration are agreed to be effective in the reproduction of animals. Although some vitamins (B group of vitamins and vitamin C) are synthesized in the organism in ruminants, fat soluble vitamins (such as vitamin A and E) must be supplied in the diet. Vitamin A is very prevalent in the plants in the form of its precursor β-carotene, rather than in the free form (Hurley and Doane, 1989 and Hemken and Bremel, 1982).

While the actual role of the E vitamin is to act as an antioxidant, it is reported to be effective on reproduction in cows as well. E vitamin is specified to be effective in the prevention of reproductive irregularities such as metritis, mastitis, retention secundarium and abortion (Hurley and Doane, 1989; Hemken and Bremel, 1982). In addition, a correlation between the follicular fluid and serum vitamin E and progesterone levels in cows has been reported (Schweigert and Zucker, 1988 and Naziroglu *et al*., 1997).

Some researchers (Hurley and Doane, 1989; Grawes-Hoagland, 1989) claim that the corpus luteum of the cow does not contain vitamin A, but plays an important role in the production of ovarium steroid hormones with the high levels of β-carotene it comprises. Animals fed with ration poor in β-carotene were reported to have lower amounts of progesterone production by the corpus luteum (Grawes-Hoagland, 1989 and Jacson *et al*., 1981). A correlation between serum and luteal tissue β-carotene level and corpus luteum progesterone secretion has been emphasized (Boos, 1987). In contrast, no significant correlation between plasma progesterone values and plasma β-carotene or liver A vitamin levels were reported in the heifers fed on baits with vitamin A and β-carotene added (Schlenzig *et al*., 1987).

There are three main roles of vitamin C, which is necessary for the reproduction of females and males. These can be listed as follows: collagen synthesis, synthesis of peptide and steroid hormones and protection of the gametes against the oxidative harms of the free radicals. Collagen synthesis is required for follicle growth and development of corpus luteum. In addition, it was stated that vitamin C applications during pregnancy reduced congenital defects and that it was also effective in stimulation of ovulation (Martin *et al*., 1995). Miszkiel *et al*. (1999) reported that vitamin C was in its lowest level in the regressed corpus luteum and in its highest level in the late luteal period; and that vitamin C and progesterone levels changed in parallel to the development and regression of corpus luteum.

The aim of this study was to investigate the serum progesterone, vitamin A, E, C and β-carotene levels and the relationship between these parameters, in pregnant and nonpregnant cows after insemination.

Materials and Methods

In this study, 17 cows at ages varying between 3-8 (6 Holstein, 7 Swiss-Brown and 4 Simental) were used. Cows having regular cycles and no clinical irregularities in the genital organs at Firat University Faculty of Veterinary Medicine, Research and Application Farm, were selected. The investigation was carried on in the months of October-December, when the animals are in stables. All the animals were kept at the same maintenance and nutrition conditions.

For synchronization of the oestrus of the cows, intramuscular application of prostaglandin F2-alpha (Estrumate, Sanofi DIF) was performed. In the following days, rectal examinations of the animals were done regularly and those which displayed oestrus were inseminated with bulls. Blood samples (10 ml) were collected into sterile blood tubes from the vena jugularis every four days starting from the insemination day, throughout an average of 21-24 days. The blood samples collected were kept at room temperature for two hours for acquirement of serum. Serums collected into centrifuge tubes were centrifuged at 3000 rpm for 15 minutes. The serums acquired were kept at -20°C in deep freeze until analysis. The pregnancy in animals were determined in between days 30-35 by B mode ultrasonography device. Subsequently, the cows were separated into two groups as pregnant and nonpregnant.

Serum progesterone levels were determined by the enzyme immunoassay method reported by Prakash *et al.* (1987). Vitamin A and β-carotene values and vitamin E levels were detected according to Suzuki and Katoh's method (1990) and Martinek's method (1964), respectively, by spectrophotometry. Blood serum vitamin C levels, on the other hand, were measured according to the phosphotungstic acid method reported by Kway (1978) by a Schimadzu UV-1208, UV-VIS spectrophotometry device.
Differences between the groups were determined according to the Unpaired Student's t-test in the statistical calculations (Hayran and Ozdemir, 1996).

Results

Twelve cows were detected to be pregnant, while 5 detected to be nonpregnant in the pregnancy examination by ultrasonography, at days 30-35 after insemination.
In the pregnant cows, serum β-carotene, vitamin A and vitamin E levels were measured to be higher compared to the nonpregnant cows (P<0.001) during the cycle and no difference was observed with regard to vitamin C levels between groups (P>0.05) (Table 1 and 2).

Table 1: Levels of serum progesteron (P4), vitamin A ve E in pregnant and non pregnant cows.

	Pregnant (n=12)			Nonpregnant (n=5)		
Days	P4 (ng/ml)	A vit. (ig/dl)	E vit. (mg/dl)	P4 (ng/ml)	A vit. (ig/dl)	E vit. (mg/dl)
0	0.56±0.05	55.74±3.15	0.208±0.01	0.38±0.07	49.52±4.69	0.176±0.02
4	1.04±0.10	54.66±3.06	0.207±0.01	0.97±0.06	49.58±4.07	0.174±0.01
8	1.85±0.12	54.43±2.87	0.203±0.01	1.82±0.21	48.56±4.16	0.178±0.03
12	2.58±0.25	52.65±2.55	0.198±0.08	1.99±0.31	46.40±3.21	0.182±0.02
16	2.46±0.19	51.91±2.98	0.200±0.01	2.40±0.37	46.62±4.16	0.188±0.01
20	2.83±0.17	51.93±3.37	0.198±0.08	1.47±0.28	47.96±3.83	0.173±0.01
21-24	2.74±0.17	50.05±3.32	0.194±0.07	0.50±0.01	45.64±4.30	0.180±0.09
ort.	2.00±0.33	53.05±0.74 [a]	0.201±0.01 [a]	1.36±0.29	47.75±0.59 [b]	0.178±0.01 [b]

(a,b): P<0.001

Table 2: Levels of serum vitamin C ve β-carotene in pregnant and non pregnant cows.

	Pregnant (n=12)		Nonpregnant (n=5)	
Days	C vit.(mg/dl)	β-caroten e(ìg/dl)	C vit.(mg/dl)	β-karoten (ìg/dl)
0	0.63±0.02	196.84±5.14	0.63±0.03	183.04±10.3
4	0.59±0.16	186.82±4.58	0.62±0.07	181.58±5.76
8	0.60±0.14	189.70±5.76	0.63±0.02	177.40±10.8
12	0.62±0.04	186.85±7.02	0.66±0.01	182.40±7.30
16	0.66±0.05 [a]	188.20±3.42	0.58±0.02 [b]	181.46±3.54
20	0.60±0.06	184.72±4.34	0.63±0.09	184.86±6.86
21-24	0.60±0.05	187.36±5.08	0.61±0.04	183.40±6.83
aveg.	0.61±0.09	188.64±1.48 [c]	0.62±0.09	182.02±0.88 [d]

(a,b): P<0.05, (c,d): P<0.001

A negative correlation between serum progesterone and vitamin E (r=-0.94, P<0.01) and progesterone and vitamin A (r=-0.89, P<0.01); and a positive correlation between vitamin A and E (r=0.95, P<0.01) was determined in the pregnant cows during the cycle. No correlation was observed in the serum progesterone, vitamin A, E, C and β-carotene levels during the cycle in nonpregnant cows.

Discussion

Average plasma vitamin A and β-carotene levels in the cows during the cycle were reported to be 33,96±1,15 and 106,75±2,75 μg/dl, respectively (Yildiz and Cay, 2002); oestrus and dioestrus vitamin A levels in cycling water buffalos and heifers were reported to be 48,22±4,79 and 60,33±3,42, respectively (Chandolia and Verma, 1987) and vitamin E levels in cows oestrus and on the 10th day of the cycle were reported to be 1.59 and 1.70 mg/ml, respectively (Naziroglu *et al.*, 1997). In winter time, plasma vitamin A and β-carotene levels on the 12th day of the oestrus cycle were stated to be 36.4 and 277.4 μg/dl in cows, respectively (Grawes-Hoagland, 1989). In our study, serum vitamin A, E and β-carotene levels detected in the pregnant and nonpregnant groups were found to be in accord with the findings of the researchers (Yildiz and Cay, 2002; Naziroglu *et al.*, 1997; Chandolia and Verma, 1987 and Grawes-Hoagland, 1989).
On day 1 and 23 of the oestrus cycle in cows, vitamin C levels were reported to be 0.39 and 0.44 mg/dl, respectively (Baspinar and Serpek, 1993) and as 5.03±0.28 μg/ml in 3 months pregnant cows in another study (Haliloglu *et al.*, 2002). The serum vitamin C levels measured in this study was found to be close to the researchers' values.
Progesterone secretion is initiated in the oestrus cycle in cows with the development of corpus luteum. The level of progesterone hormone, which peaks at the luteal period, decreases in the last days of the cycle if no pregnancy occurs (Yildiz, 2000 and Christensen *et al.*, 1974). While it rises to its highest level in the pregnant cows on day 20 after insemination, an increase in between days 4-16 was detected in nonpregnant cows, which was followed by a reduction later on.
Plasma vitamin E levels measured every 10 days in between days 8-58 after insemination was reported to be higher in the pregnant cows, compared to the nonpregnant cows (Aslan *et al.*, 1998). Aksakal *et al.* (1995) stated that the plasma vitamin E levels (4.31±0.27, 1.64±0.12 μg/ml) of pregnant cows, measured in the months of June and December was higher (P<0.05) than the plasma vitamin E levels of cows in oestrus (3.44±0.16, 1.12±0.03 μg/ml). In the study performed, it was observed that there was no difference between the

sampling days during the cycle in pregnant and nonpregnant cows (P>0.05), but that the average serum vitamin E levels during the cycle were higher in pregnant cows than that of the nonpregnant cows.

It was reported that on days 0, 7 and 23 following the insemination in cows, the plasma vitamin C level was higher in pregnant cows compared to the nonpregnant cows (Semacan *et al.*, 2002). In this study, a higher vitamin C level observed on day 16 following insemination in pregnant cows compared to the nonpregnant cows can be due to vitamin C's functioning as a cofactor in the hydroxylation reactions in the biosynthesis of steroid hormones (Martin *et al.*, 1995; Semacan *et al.*, 2002). There was no difference in the average serum vitamin C levels between the groups during the cycle. The findings related to vitamin C was observed to be similar to those of Semacan *et al.* (2002).

Aksakal *et al.* (1995) reported that there was no difference between the plasma β-carotene levels of pregnant cows (2-3 months pregnant) during the month of June (7.38±0.32 μg/ml) and plasma β-carotene levels of those in the luteal phase of the cycle (8.00±0.30 μg/ml); but that the corpus luteum β-carotene levels in the month of December (2.42±0.14 μg/ml) was higher (P<0.05) in the pregnant cows, compared to the corpus luteum β-carotene levels of the cows in the luteal phase (1.56±0.29 μg/ml). It was reported that the β-carotene levels in the blood plasma collected every other day until day 23 after the insemination was higher (P<0.01) in the pregnant cows compared to the cycling cows, but that there was no difference with regard to vitamin A between the related groups and that it would be useful to feed the cows bred in closed stables with baits rich in β-carotene, to increase the fertility rate (Haliloglu *et al.*, 2003). In some other studies performed (Aslan *et al.*, 1998 and Bhuvnesh *et al.*, 2001), it was stated that plasma vitamin A and β-carotene levels were higher in pregnant cows and heifers compared to the nonpregnant ones and that these parameters might be influenced by the physiological state of the animals. In this study, the average serum β-carotene and vitamin A levels were detected to be higher in the pregnant cows during the cycle compared to the nonpregnant cows. Our findings are in accord with the results of some other researchers (Aslan *et al.*, 1998; Bhuvnesh *et al.*, 2001 and Haliloglu *et al.*, 2003).

An increase in the serum vitamin E, vitamin A and β-carotene levels and a decrease in the progesterone levels on from the insemination day was detected in pregnant cows. This reduction in the vitamin and β-carotene levels is thought to be effective on the development of corpus luteum which provides the continuation of pregnancy and on the initiation of the progesterone synthesis, as the vitamin E and β-carotene levels in corpus luteum in the luteal phase is reported to be higher compared to the other periods of the cycle (Schweigert, 2003).

It is claimed that there is a positive correlation between serum β-carotene levels and progesterone values and a negative correlation between serum vitamin A and progesterone levels in cows (Grawes-Hoagland *et al.*, 1989). In another study performed with the follicle fluid of simmental cows (Schweigert and Zucker, 1988), a negative correlation between progesterone and vitamin A and a positive correlation between progesterone and vitamin E and β-carotene and vitamin E was detected. It is stated that there is a negative and significant correlation between serum vitamin E and progesterone levels during the cycle (Naziroglu *et al.*, 1997). Cassano *et al.* (1999) emphasized that there was a correlation between the follicle fluid and plasma vitamin A and E levels. In the pregnant cows, a negative correlation between serum progesterone and vitamin E (r=-0.94) and progesterone and vitamin A (r=-0.89); a slightly negative correlation between progesterone and β-carotene (r=-0.74); and a positive correlation between vitamin E and A (r=0.95) during the cycle was observed. No correlation was detected between these parameters in the nonpregnant animals. Our findings are in accord with the results of researchers (Schweigert and Zucker, 1988; Naziroglu *et al.*, 1997; Cassano *et al.*, 1999 and Grawes-Hoagland *et al.*, 1989).

As a conclusion, the fact that the average blood serum vitamin A, E and_β-carotene levels in pregnant cows after insemination are higher compared to the cycling cows may be effective in progesterone secretion by corpus luteum, which ensures the continuation of pregnancy. In addition, significant correlations between progesterone and vitamin A, progesterone and vitamin E and vitamin A and vitamin E during the cycle were detected.

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