Original article

The Effect of Some Parameters in the Blood on Reproductive Efficiency in Dairy Cows During Insemination

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Abstract. *Study Objectives:* Infertility is one of the common factors that negatively affect productivity and quality in dairy farms. The study aimed to determine the levels of nitric oxide, total protein, glucose, vitamin C, cholesterol, urea nitrogen, MDA, and carotene during insemination and the relationship between these values and fertility. For this purpose, a total of 80 Holstein cows were studied. *Methods:* Non-pregnant cows whose pregnancies were diagnosed by rectal examination were separated for the study and synchronization was applied. Blood samples were taken from the vena jugularis at the same time 12 hours after insemination and sent for analysis. After the cows were detected by ultrasound on the 30th day, the relationship between the blood values of the pregnant and non-pregnant cows with the pregnancy rate was investigated. *Results:* Pregnancy rates were determined as (70%) as a result of the research. When the comparison of biochemical parameters obtained from the study according to pregnancy status was examined, it was seen that there was no statistically significant difference in all other data except for glucose level (69.53 mg /dl pregnant, 153.12 mg /dl not pregnant). *Conclusion:* Infertility rate (30%) has been associated with MDA (7.92 mmol / L in pregnant cows, 9.00 mmol / L in non-pregnant cows), an indicator of oxidative stress in addition to β-carotene (71.26 μg / dl in pregnant, and 93.54 μg /dl in nonpregnant) deficiency, high glucose, and BUN (pregnant 21.56 mg /dl, non-pregnant 22.63 mg /dl) levels.

Key words: Pregnancy, Blood parameters, Reproductive, Dairy cows

Introduction

Vitamins and minerals are essential for the biological development and reproduction of animals. Plasma concentrations of β-carotene have a marked effect on reproduction. The use of carotene in reproductive performance in livestock is not fully explained, its effect has been attributed to uterine modification and its role in the reproductive hormone release mechanism (1-3). The antioxidant effect of β-carotene, the precursor of vitamin A and influences on reproductive functions are documented.

One mg of β-carotene is parity to 400 IU of vitamin A. Many reproductive disorders have been recorded due to β-carotene deficiency in cows i.e. delayed ovulation, increases abortions and early embryonic loss rates, silent oestrus, increase tendencies of ovarian cysts, Retention of fetal membranes, and metritis risk, abort or weak birth, and blind calves and reduces fertility, etc. The daily requirement of a cow is 0.18 mg of β-carotene/kg body weight in order to maintain its normal reproductive functions (4). While β-carotene is used as a source of vitamin A in the ovaries, it is involved in rupturing the follicle membrane during ovulation. In addition, there is a positive correlation between carotene levels in plasma both follicular fluid, luteal tissue, and the corpus luteum (5). The corpus luteum contains a high level of β-carotene. High β-carotene levels influence the release of ovarian steroids. The effect of β-carotene on the secretion of LH from the hypothalamus has also been observed by other researchers and thus increased progesterone production. The normal level of β-carotene in serum of dairy cows are 300-1200 μg/100 ml, subnormal level 100-200 μg/100 ml, and level of 9-100 μg/100 ml is regarded as deficiency (4).

Vitamin C is a water-soluble antioxidant. It is not an essential nutrient for adult dairy cattle due to the biosynthesis of cattle in their own body. Cattle are susceptible to ascorbic acid deficiency because exogenous sources of this vitamin are rapidly destroyed by the rumen microflora. The importance of vitamin C in dairy cattle reproduction neutralizes oxidation by cellular respiratory products in luteal cells and is an enzymatic cofactor in collagen synthesis as a promoter of steroid and protein hormone synthesis (3).

Cholesterol is a type of fat that is essential for life in the bloodstream. It is necessary to form, maintain important parts of cells and to make for several essential hormones. Raised cholesterol doubles the development of cardiovascular disease, diabetes mellitus, and reproductive disorders. The close relationship of blood glucose level with fertility has been emphasized in studies. It is reported that the serum luteal hormone wave decreases in the postnatal period, and this is shaped by the cystic ovary, ovulation delay, anovulation, and insufficient luteal structure. Researchers have reported that glucose is necessary and that there should be a certain limit value (6, 7). Glucose international values are stated as non-pregnant 34.79-69.11 mg /dl, pregnant 31.6 - 74.1 mg / dl (8).

Urea is an organic molecule found in blood and fluids. In dairy cows, the blood urea nitrogen (BUN) value is a nutritional biomarker in protein. Increasing BUN height in cows fed rations containing high protein content negatively affects fertility. Uterine pH shifts to one side, which causes early embryonic deaths (9, 10).

Nitric oxide (NO) is a highly diffusible molecule and is an important modulator of female reproductive functions, including folliculogenesis oocyte maturation, fertilization, and implantation. NO synthase (NOS) deficiency or the presence of its inhibitor has been associated with impaired ovulation, fertilization failure, and implantation failure (11-14). Korzekwa et al. (15), stated *in vivo* and *in vitro* studies that nitric oxide (NO) undergoes luteolysis in cattle. However, it suggested that nitric oxide, produced locally in the bovine corpus luteum (CL), inhibits progesterone (P4) synthesis and is a component of the luteolytic induced by uterine prostaglandin (PGF2α) (16).

Maintaining homeostasis in cells is the result of a complex interaction between prooxidants and antioxidants. Oxidative stress occurs as a result of this interaction between prooxidants and antioxidants shifting towards excessive free radical formation. Oxidative stress affects all important components of cells, including lipids, proteins, carbohydrates, and DNA. One of the most important harmful effects of free radical attack is the oxidation of unsaturated fatty acids, known as lipid peroxidation. One of the end products of lipid peroxidation is malondialdehyde (MDA) (13, 17). Serum MDA levels in healthy cattle were reported as 1.74 ± 0.25 nmol / mL (18). In this study, nitric oxide (NO), MDA, total protein, glucose, vitC. It was aimed to investigate the effects of chemical markers such as cholesterol, urea nitrogen, and carotene on fertilization success.

Materials and Methods

Materials

In the study, intensive care and operating instructions and modern herd management systems were provided in Balikesir province, and a special dairy breeding cow farm housed 80 dairy cows. Studies were conducted on 40 cows between the ages of 3-5, who were artificially inseminated by the technical staff of the enterprise 60 days after birth and whose oestrus did not return 18-24 days after insemination. The mean daily milk production per cow was close to 26 kg during the study period. There were no clinical endometritis, mastitis, and laminitis in the cows (Ethics committee approval 2021/1-3).

Method: Pregnancy diagnosis, synchronization, and insemination

In the animals used in the study, the ovarian activities were determined primarily by performing a rectal examination. Non-pregnant animals with active corpus luteum in one of their ovaries were separated for the synchronization of their cycles. Animal cycles were synchronized with PGF2α Animals showing oestrus after the first injection were inseminated. The animals that did not show oestrus were included in the study by injecting the second PGF2α after 11 days and inseminating the animals showing oestrus. Oestrus was determined by the activity meter and personnel observation methods used in the dairy farm. Animals were inseminated 12 hours after the first signs of oestrus were observed. Inseminations made with bull semen whose fertility was previously known and used in the farm were carried out by the farm veterinarian each time. Pregnancy status was determined by performing ultrasonographic examination to inseminated animals 30 days after insemination. The examination was performed with a real-time B mode ultrasound with a 6-8 MHz transrectal probe. As a result of the examination, pregnant and non-pregnant animals were determined, divided into two groups, and evaluations were made over these groups.

Taking the blood samples

At the time of insemination from the animals included in the study, the neck part was disinfected, and 10 ml of blood was taken from the vena jugularis into sterile glass tubes with anticoagulant, and the blood samples were transported to the laboratory within 2 hours and centrifuged at 3000 rpm and their plasma was removed. The taken plasmas were stored in a -20-degree freezer until the analysis was performed.

Performing Ultrasound Examinations

In the examination, the device (Hasvet-shenzhen well. d wed-3000v, China) has a B mode, 7.5 MHz frequency, transrectal linear probe.

In the ultrasonic examination, the quality of the ovary note CL found in rectal examination (large> 22 mm, luteal tissue density, compactness or not) was determined. Likewise, the other ovary was also evaluated. In this way, when ultrasound pregnancy diagnosis emerged in scanning the uterus, the uterine horns where CL was found were revealed. In animals without quality CL in one of the ovaries (not insufficient size), one of its cornu uteri was scanned in detail in terms of possible pregnancy in longitudinal and transversal sections starting from the bifurcation area. The animal was accepted as pregnant when at least 3 of the following findings were observed in the scanning of the corneous uterus. These are amniotic fluids, fetal membranes, umbilical cord, fetal heartbeat, fetus itself, and compartmental structures in the uterus. The animals whose symptoms were not observed were considered as non-pregnant.

Some parameters Determination in blood samples

Blood samples were sent to the System Laboratory, Department of Physiology to determine the levels of NO, cholesterol, total protein, glucose, vitamin C, MDA, urea-nitrogen, and beta-carotene in plasma.

Serum blood urea nitrogen (BUN), creatinine, total protein, albumin, total bilirubin, GGT, LDH, AST, ALP, CK, Ca, P, Mg, glucose, cholesterol, triglyceride concentrations with commercial kits and autoanalyzer (BT 3000 plus, Roma, Italy).

For the analysis of vitamin C, the HPLC analysis method consisting of a spectrophotometer detector and a reversed phase column was carried out (19).

Nitric oxide level was determined according to the method stated by (20). The principle of the test is based on the diazotization of nitrite with a primary aromatic amine (sulfanilamide) in an acidic environment and forming a colored azo derivative with N- (1-naphthyl) ethylenediamine (NEDD) (Griess reaction). The resulting compound is a purple azo dye that can absorb light at a wavelength of 545-555 nm.

Determination of MDA from peroxidation products resulting from free radicals was determined using the double boiling method of (21).

For the determination of plasma beta carotene level, the spectrophotometric method defined by (22) based on the separation of beta carotene with the addition of hexane from plasma lipoproteins precipitated with ascorbic acid was used

Statistical analysis

The normal distribution test of biochemical parameters obtained from the study according to the groups was performed by Kolmogorov-Smirnov and Shapiro Wilk tests. It was determined that all variables except the glucose variable showed normal distribution according to the groups ($p > 0.05$). After testing the normality distribution assumption, the results were presented as mean and ± standard deviation (SD) or as the median and interquartile difference (IQR). The t-test in comparison of variables providing normal distribution assumption according to gestational condition, normal distribution. The Mann-Whitney U test was used to compare variables that did not provide assumptions according to pregnancy status. Analyzes were carried out with the help of the SPSS 22.0 program. $p<0.05$ significance level was chosen.

Table 1. Pregnancy status of animals after insemination

Groups	Number	Percentage (%)
Pregnant	28	70.0
Non-pregnant	12	30.0
Total	40	100.0

Results

Biochemical parameters obtained from 28 pregnant (70%), and 12 non-pregnant (30%) animals were used in the study (Table 1). After testing the normality distribution assumption, the results were presented as mean and ± SD or as median and IQR.

When the comparison of biochemical parameters obtained from the study according to pregnancy status was examined, it was determined that there was no statistically significant difference ($p > 0.05$). Statistical mean values and averages of the comparison of all variable groups according to their gestational status were stated (Table 2).

Discussion and Conclusion

The maintenance and optimization of reproductive system activity in dairy and livestock herds around the world, especially practices to increase milk production have become an important issue. Modern dairy industries often put cows' productive abilities at risk due to metabolic disorders. Physiological and

Table 2. Comparison of biochemical parameters according to pregnancy status

Variables	Group	N	Mean	Standard deviation (SD)	t	p
NO (mmol/mL)	Pregnant	28	11.23	4.60	0.072	0.943
	Non-pregnant	12	11.12	3.99		
CHOLESTEROL (mg/dl)	Pregnant	28	110.04	42.15	-0.812	0.422
	Non-pregnant	12	121.32	35.22		
PROTEIN (g/dl)	Pregnant	28	3.44	1.25	1.125	0.268
	Non-pregnant	12	3.01	.62		
VİT C (μg/dl)	Pregnant	28	10.79	4.13	-0.770	0.446
	Non-pregnant	12	11.81	3.03		
MDA (mmol/L)	Pregnant	28	7.92	2.34	-1.303	0.200
	Non-pregnant	12	9.00	2.56		
UREA (mg/dl)	Pregnant	28	21.56	6.89	-0.470	0.641
	Non-pregnant	12	22.63	5.70		
CAROTENE (μg/dl)	Pregnant	28	71.26	32.67	-2.007	0.052
	Non-pregnant	12	93.54	30.94		
		N	Median	IQR	Z	p
GLUCOSE (mg/dl)	Pregnant	28	69.53	11.59	-1.505	0.138
	Non-pregnant	12	153.12	154.25		

biochemical parameters must be determined in order to predict such disorders. Therefore, metabolic profile tests can help identify biomarkers that can affect reproductive or productive performance (8). Therefore, some serum biochemical markers in the study nitric oxide, total protein, glucose, vitamin C, cholesterol, urea nitrogen, MDA, and carotene levels were examined.

It was found that the blood NO levels in the study did not differ significantly between pregnant and non-pregnant cows (pregnant 11.23 mmol / mL ± 4.60 and non-pregnant 11.12 mmol / mL ± 3.99) and thus did not affect pregnancy. The results of the study were similar to the study of (14). In the study, it was stated that there was no significant difference in the NO level between cows who were pregnant and non-pregnant cows during the sampling days (14).

In cows, there is an important relationship between cholesterol concentration during the oestrus cycle and the probability of fertilization and pregnancy development. Often in the postpartum period, high cholesterol levels are associated with its increased use for milk production. Differences in dietary fat sources can cause differences in circulating cholesterol levels (23). There are no significant differences in blood cholesterol levels between pregnant and non-pregnant cows in the study (pregnant 110.04 mg / dl, non-pregnant 121.32 mg / dl). According to (23), the results of the study coincided with differences in pre-fertilization (5.83 mmol / L ± 0.18) and pre-calving (3.14 mmol / L ± 0.17) cholesterol levels. Healthy cows under normal feeding and grooming conditions are characterized by a decrease in blood cholesterol level during pregnancy and a noticeable increase in the first months of lactation (24). The blood cholesterol values of the study were also measured by (25) coincide with the results. Although there were no significant differences between the pregnant and non-pregnant cows of the 2 breeds in the study, 208.77 mg / dl ± 4.79 in the zebu breed, and the pregnant animals of the same breed, the blood cholesterol level decreased during pregnancy 201.11 mg / dl ± 3.80. Reference values for some frequently measured variables in the blood are compiled from the clinical laboratories of the Western College of Veterinary Medicine at the University of Saskatchewan. These values were determined at the cholesterol level as 65–220 mg / dl international values (26).

According to Oliveira et al. (2020) study, it was determined that there was no difference in total protein values between the breeding periods of cows (p>0.05) (27). Total protein international values non-pregnant 5.78 – 10.03 g/dl, pregnant 4.96 - 10.24 g/dl (8). The protein level in the study (pregnant 3.44 g / dl and non-pregnant 3.01 g / dl, p> 0.05) was found to be insufficient. According to Abdullah et al. (2017) study, although there were no significant differences between the pregnant and non-pregnant cows of the 2 races, the cross breed was 6.12 mg / dl ± 0.26 in the non-pregnant, 6.59 mg / dl ± 0.21 in the pregnant, and 6.42 mg / dl ± 0.21 in the 6.95 mg / dl ± 0.32 values were determined (25). In the study, there was no significant difference between the total protein values between pregnant and non-pregnant animals, which is consistent with the results.

Abdullah et al. (2017), although there was no significant difference between the pregnant, and non-pregnant cows of the two breeds, the crossbred race was 28.18 mg / dl ± 1.8 in non-pregnant, 35.53 mg / dl ± 2.2 in pregnant, and 26.68 mg / dl ± 1.89 in non-pregnant in the zebu race values of ± mg / dl 3.20 were determined. It has been noted that higher protein intake can lead to a high plasma urea nitrogen concentration because during the experimental period animals were fed high protein legumes, which may contribute to high BUN (25).

As a result of the research, international BUN values were determined as 11.51 - 30.64 mg / dl for non-pregnant, 14.07 - 31.22 mg /dl for pregnant (8). In another study, it was reported that the higher the urea nitrogen levels in body fluids above a certain value, the lower the pregnancy rates (9). With this information, the study results were determined to be within international limits (pregnant 21.56 mg /dl, non-pregnant 22.63 mg / dl). Each 1 mg / dl increase in BUN concentrations causes an 8% decrease in the pregnancy rate. In general, it is stated that BUN values in pregnant cows are significantly lower than BUN values in non-pregnant cows (9). Kenny et al. (2002) reported that early embryo viability and rate were not affected by less than 25 mmol / L (70.028 mg / dl) BUN, therefore environmental effects of the early embryonic period were affected very little (28).

The overall mechanism of β-carotene is not clearly understood and there are still controversies about its effect on reproduction. While some reports point out β-carotene has a positive effect there are also reports indicating it has no or negative effect on reproductive parameters in cows. Besides this information, the amount of carotene required to maintain normal reproduction indicates a higher requirement (60 to 198 μg/kg) than those reported for other functions (29). The normal level of β-carotene in the blood of dairy cows is 300-1200 μg/100 ml, subnormal level 100-200 μg/100 ml and 9-100 μg/100 ml is described as deficiency (4). According to Ataman et al (2010) study, β-carotene serum concentrations were not related to the incidence of retained placental fetal membranes, endometritis, ovarian cysts or the onset of cyclicity post-partum and the authors suggest that there is only a minor relationship between the β-carotene serum (3). In this study, the amount of carotene is between 25-136 μg / dl, which is similar to its low level in the study (blood β-carotene in the study was found to be 71.26 μg / dl in pregnant and 93.54 μg / dl in non-pregnant). This study found that no significant differences in blood carotene levels in pregnant and non-pregnant cows. In addition, the proportion of non-pregnant (30%) in the study results can be associated with carotene deficiency. Researchers found that cows with greater beta-carotene concentration had fewer pregnancy losses and more pregnancies per AI compared to cows with lower serum beta-carotene concentrations. In this study, blood samples were not collected or analyzed at different time points for progesterone in early pregnancy, so it is unclear whether beta carotene affects progesterone concentrations (30).

Kadhim and Ali (2019) reported that in dairy cows, glucose concentration is low as it is mostly produced in the process of gluconeogenesis and large amounts are secreted in milk as a form of lactose (8). According to Macmillan et al. (2020) Serum concentrations of Ca, P, K, Na, Cl, total protein, albumin, globulin, urea, glucose, GGT, GLDH, and BHB were not found to be different between pregnant and non-pregnant cows following first AI. The study results are also consistent with the high glucose level in non-pregnant cows (pregnant 2.80 mmol / L, non-pregnant 2.90 mmol / L) (31). According to the research of Oliveira et al. (2020) the highest glucose level (72.88 mg / dl) in non-pregnant cows, while showing low glucose levels in the early-middle and late stages of gestation. It has been reported that the lowest glucose level between gestational periods is in the late period of pregnancy (49.44 mg / dl) (27). Study results are consistent with non-pregnant cows with high glucose levels (non-pregnant 153.12 mg/dl and pregnant 69.53 mg/dl). Also in another study, the blood glucose and the plasma non-esterified fatty acids (NEFA) and insulin concentrations were estimated in jugular blood samples from goats during pregnancy and compared with samples from non-pregnant goats and from goats during the periparturient period. Also in another study, blood glucose and plasma NEFA and insulin concentrations were determined in jugular blood samples from goats during pregnancy and compared with samples from pregnant and non-pregnant goats during the periparturient period. Blood glucose levels in non-pregnant goats were significantly ($p < 0.01$) higher than in pregnant goats except days 42 to 70 (59 ± 1.36 mg / ml) (32). In another study, it was reported that cows with increased calving up to conception interval (> 150 days of conception) had higher plasma glucose levels compared to cows conceiving within 150 days. Slower glucose clearance and increased insulin resistance are associated with the development of ovarian cysts in postnatal cows. Plasma glucose was higher in cows with clinical endometritis compared to healthy ones. Our results are consistent with the author's results with higher pre-term postnatal glucose concentrations in cows with reduced fertility (33).

In the study by Ataman et al. (2010) there were no significant difference in pregnant and non-pregnant cows' vitamin C levels at the time of and 21 days after AI between However, mean vitamin C levels at the time of artificial insemination were higher in non-pregnant cows than in pregnant cows (pregnant cows 2.85 μg / ml ± 0.17 and non-pregnant non-pregnant 2.96 μg / ml ± 0.50) (3).

The administration of vitamin C during the period of hormonal stimulation in dairy cows has shown a positive though the statistically insignificant impact in terms of a higher number of pregnancies. However, the exact mechanism by which vitamin C supplementation improves breeding performance in cattle remains inconclusive (34).

Colakoglu et al. (2017) reported that plasma MDA level was significantly higher in early lactating cows compared to advanced pregnant cows. In their study, MDA levels of the winter group and the summer group were 3.69 and 4.1 µmol / L on day 0, respectively, while the MDA levels of the two groups on day 21 ranged from 3.3 to 3.4 (17). The results obtained in this study showed higher oxidative stress and lower antioxidant activity due to the effect of lactation and calving in the postnatal period. While blood antioxidant activity was negatively correlated, oxidant activity showed a positive correlation with fertility parameters (13, 17). In particular, the negative effects of oxidative stress on the reproductive system include damage to oocyte DNA, ovaries, and endometrium, and this has implications for fertility. MDA showed a positive correlation with Glucose (35). In the study, during insemination (early lactation period), MDA value negatively affected fertility due to oxidative stress (7.92 mmol / L in pregnant cows, 9.00 mmol / L in non-pregnant cows). A positive correlation was found between glucose and MDA levels.

In conclusion, no significant difference was observed in blood values between pregnant cows and non-pregnant cows in this study. 30% infertility rate can be associated with a β-carotene deficiency in the blood, high glucose, BUN level, and high MDA, which is an indicator of oxidative stress. Studies on the pathophysiology of unexplained infertility (20-25%) have shown that oxidative stress may be an underlying factor (13). Additionally, more research is needed to evaluate these parameters and their effects on bovine reproduction.

References

1. Yıldız H, Kaygusuzoğlu E, Kızıl Ö.Serum progesterone, vitamin A, E, C and beta-carotene levels in pregnant and non pregnant cows post-mating. Bull. Vet. Inst. Pulawy 2005; 49: 199-202.
2. Ayaşan T, Karakozak E. Hayvan beslemede β-karoten kullanılması ve etkileri. Kafkas Univ Vet Fak Derg, 2010; 16(4): 697-705.
3. Ataman MB, Erdem H, Bülbül B, Haliloğlu S, Çınar M, Aköz M. Plasma β-carotene, vitamin A and vitamin C levels in cyclic and pregnant cows. Kafkas Univ Vet Fak Derg, 2010; 16(4): 579-584.
4. Bhatnagar PC, Choudhary JL, Bhardwaj B, Sharma CSDK. Synergic effect of β-carotene in reproductive functioning of dairy cows. Journal of Entomology and Zoology Studies, 2020; 8(1): 700-702.
5. Yeşil M, Sarıözkan S. Dişi üreme sistemi açısından önemli bazı vitamin ve mineraller. Erciyes Üniversitesi Veteriner Fakültesi Dergisi, 2017; 14(3): 201-208.
6. Salmanoğlu R, Baştan A, Salmanoğlu B, Küplülü Ş, Vural MR. Çeşitli fertilite problemli Holştayn ırkı ineklerde kan beta karotin, retinol glikoz ve kolesterol düzeyleri. Ankara Üniversitesi Veteriner Fakültesi Dergisi, 1997; 44(2-3): 151-157.
7. Güzel S, Tanriverdi M.Comparison of serum leptin, glucose, total cholesterol and total protein levels in fertile and repeat breeder cows. Revista Brasileira de Zootecnia, 2014; 43(12), 643-647.
8. Kadhim M, Ali B. Levels of some biochemical markers in sera of pregnant and non-pregnant lactating dairy cows in Baghdad, Iraq. Iraqi Journal of Veterinary Sciences, 2019; 33(2): 353-358.
9. Aydın İ. Sığırlarda kan üre nitrojen düzeyinin fertiliteye etkisi. Erciyes Üniversitesi Veteriner Fakültesi Dergisi, 2007; 4(1): 49-56.
10. Ayaşan T. Süt ineklerinin beslenmesinde süt üre nitrojenin önemi. Gaziosmanpaşa Univ Zir Fak Derg, 2009; 26(2): 27-33.
11. Lee TH, Wu MY, Chen MJ, Chao KH, Ho HN,YangYS. Nitric oxide is associated with poor embryo quality and pregnancy outcome in *in vitro* fertilization cycles. Fertility and Sterility, 2004; 82(1): 126–131.
12. Özen A, Ergün E, Öztaş E, Ergün L, Özcan Z, Alabay B, Bayraktaroğlu AG, Kürüm A, Erdoğan E. Farklı siklus dönemlerinde inek oviduktun da nitrikoksit sentaz enzimlerinin (iNOS, eNOS, nNOS) immuno histokimyasal olarak gösterilmesi. Ankara Üniversitesi Bilimsel Araştırma Projesi Kesin Raporu. Ankara 2011; pp. 43.
13. Yalçınkaya E, Çakıroğlu Y, Doğer E, Budak Ö, Çekmen M, Çalışkan E. Effect of follicular fluid NO, MDA and GSH levels on *in vitro* fertilization outcomes. Journal of the Turkish German Gynecological Association, 2013; 14(3): 136.
14. Karen A, Bajcsy AC, Minoia R, Kovács R, De Sousa NM, Beckers JF, Tibold J, Madl I, Szenci O. Relationship of progesterone, bovine pregnancy-associated glycoprotein-1 and nitric oxide with late embryonic and early fetal mortalities in dairy cows. Journal of Reproduction and Development, 2014; 2013: 033
15. Korzekwa AJ, Okuda K, Wloclawek-Potocka I, Murakami S, Skarzynski DJ. Nitric oxide induces apoptosis in bovine luteal cells. Journal of Reproduction and Development. 2006; 52(3): 353-361.
16. Shirasuna, K. Nitric oxide and luteal blood flow in the luteolytic cascade in the cow. Journal of Reproduction and Development, 2010; 56(1): 9-14.
17. Colakoglu HE, YazlikMO, Kaya U, Colakoglu EC, Kurt S, Oz B, Bayramoğlu R, Vural MR, Kuplulu S. MDA and GSH-Px activity in transition dairy cows under seasonal

variations and their relationship with reproductive performance. Journal of Veterinary Research, 2017; 61(4): 497-502.
18. Ercan N, Tuzcu N, Başbuğ O. The evaluation of important biomarkers in healthy cattle. İnflammation, 2014; 3(7): 8.
19. Haiying L, Padilla L, Yoshimatsu K, Matsui T, Kitagawa M, Yano H. Determination of plasma vitamin C concentration in fattening cattle. Animal Science Journal, 2003; 74(1): 7-10.
20. Miranda KM, Espey MG, Wink DA. A rapid, simple spectrophotometric method for simultaneous detection of nitrate and nitrite. Nitric oxide, 2001; 5(1): 62-71.
21. Draper HH, Hadley M. [43] Malondialdehyde determination as index of lipid Peroxidation. Methods in enzymology, 1990; 186: 421-431.
22. Suzuki J, Katoh N. A simple and cheap methods for measuring serum vitamin A in cattle using only a spectro-photometer. Jpn Vet Sci, 1990; 52 (6): 1282-1284.
23. Vasilenko TF. Multi directional changes in the blood cholesterol in mammals of different species during pregnancy and lactation. IJSBAR,2016; 30(2): 59-70.
24. Василенко ТФ. Factors determining the elevated blood cholesterol level in dairy cows during first month of months of postpartum. Проблемыбиологиипродуктивныхживот ных, 2020;2: 75-81.
25. Abdulah M, Mohanty TK, Bhakat M, Kumaresan A, Patbandha TK, Madkar AR, Mohanty AK. Metabolic indicators for early pregnancy in zebu and crossbred dairy cows reared in a subtropical climate. Turkish Journal of Veterinary and Animal Sciences, 2017; 41(3): 407-413.
26. Constable PD, Hinchcliff KW, Done SH, Grünberg W. Veterinary Medicine-e-book: A textbook of the Diseases of cattle, horses, sheep, pigs and goats. Missouri: Elsevier Health Sciences, 2016; 2218-2219.
27. Oliveira WDCD, Bezerra LR, Araújo MJD, Edvan RL, Oliveira RL. Protein, energetic, enzymatic and mineral profile of Nellore cows during the pregnancy, parturition and postpartum. ActaScientiarum. Animal Sciences, 2020; 42, 2-9.
28. Kenny DA, Humpherson PG, Leese HJ, Morris DG, Tomos AD, Diskin MG, Al KET. Effect of Elevated Systemic Concentrations of Ammonia and Urea on the Metabolite and Ionic Composition of Oviductal Fluid in Cattle 1, Biology and Reproduction, 2002; 66(6): 1797–1804.
29. Hemken RW, Bremel DH.Possible role of beta-carotene in improving fertility in dairy cattle. J Dairy Sci, 1982; 65: 1069-1073.
30. Madureira AML, Pohler KG, GuidaTG, Wagner SE, Cerri RLA, Vasconcelos JLM. Association of concentrations of beta-carotene in plasma on pregnancy per artificial insemination and pregnancy loss in lactating Holstein cows. Theriogenology, 2020; 142: 216-221.
31. Macmillan K, Gobikrushanth M, Helguera I, Behrouzi A, Colazo MG. Relationships between early postpartum nutritional and metabolic profiles and subsequent reproductive performance of lactating dairy cows. Theriogenology. 2020; 151: 52–57.
32. Khan JR, Ludri RS. Changes in blood glucose, plasma non-esterified fatty acids and insulin in pregnant and non-pregnant goats. Tropical Animal Health and Production, 2002; 34(1): 81–90.
33. Bicalho MLS, Marques EC, Gilbert RO, Bicalho RC. The association of plasma glucose, BHBA, and NEFA with postpartum uterine diseases, fertility, and milk production of Holstein dairy cows. Theriogenology, 2017; 88: 270-282.
34. Ranjan R, Ranjan A, Dhaliwal GS, Patra RC. L-Ascorbic acid (vitamin C) supplementation to optimize health and reproduction in cattle. Veterinary Quarterly, 2012; 32(3-4): 145-150.
35. Boudjellaba S, Ainouz L, Tennah S, Temim S, Iguer-Ouada M. Reproduction performance and blood biochemical parameters in dairy cows: Relationship with oxidative stress status. Veterinary World, 2018: 11(6); 883.

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