Original Article

Pre- and Post-partum Serum Concentration of Adiponectin, Leptin, and Ghrelin and Their Ability to Predict the Reproductive Performance and Milk Production Indexes in Holstein Dairy Cows

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ABSTRACT

Background: Adiponectin, leptin, and ghrelin are metabolism regulatory factors affecting milk production and reproductive performance.

Objectives: This study aimed to investigate the adiponectin, leptin, and ghrelin serum concentration in predicting Holstein cows' reproductive and milk production indexes in the post-partum period.

Methods: In this research, 45 clinically healthy and pregnant Holstein dairy cows were randomly selected near the forthcoming calving. Blood samples were taken from the jugular veins of cows 15 days before and after parturition, and serum concentrations of adiponectin, leptin, ghrelin, and some other metabolic biochemical parameters were measured. The animals were monitored for milk production and reproductive parameters for 3 consecutive months.

Results: Findings showed no significant difference in adiponectin concentration between the pregnant and non-pregnant cows following the first artificial insemination (AI); however, pre- and post-partum serum concentrations of leptin, ghrelin, and insulin were higher in conceived cows following the first AI (P<0.05). A significant negative correlation was found between post-partum serum adiponectin concentration, the number of services before conception, and mean milk production levels (P<0.05). However, adiponectin did not have a long-lasting effect on the reproductive and milk production indexes because of other studied variables.

Conclusion: It was concluded that the post-partum increased serum adiponectin and ghrelin and decreased leptin concentrations could not predict the reproductive and milk production indexes.

Keywords: Adiponectin, Ghrelin, Leptin, Milk production indexes, Reproductive indexes

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1. Introduction

Feed intake is an important factor in preventing metabolic stress in a transitional period, defined as 3 weeks before and after the parturition of cows (Sordillo & Raphael, 2013). During the transitional period, nutritional requirements increase rapidly along with fetal growth and milk production, consequently inducing negative energy balance (NEBAL), which extends 8-10 weeks (Lean et al., 2013). NEBAL delays the timing of first ovulation through attenuation of Luteinizing hormone (LH) pulse frequency and low levels of blood glucose, insulin, and insulin-like growth factor I that collectively limit estrogen production by dominant follicles (Lean et al., 2013). Dairy cattle experience appetite suppression during the last week of gestation before dry matter intake recovers, and it can take up to a few weeks after calving. Therefore, appetite-determining factors can affect feed intake and the presence of periparturient metabolic disorders (Lean et al., 2013; Sordillo & Raphael, 2013). Concurrent with the transitional period, a state of insulin resistance may develop as a part of physiologic (late pregnancy and early lactation) or pathologic processes that may appear as a reduced insulin sensitivity or decreased insulin responsiveness (Azarbayejani & Mohammadsadegh 2021; De Koster & Opsomer, 2013) and an increased insulin concentration (hyperinsulinemia) (Artunc et al., 2016). During this period, glucose consumption is reduced in many tissues to supply sufficient glucose for fetal growth and to produce colostrum and milk (De Koster & Opsomer, 2013).

Severe insulin resistance, especially in overweight dairy cows, may contribute to excessive adipose tissue lipolysis (Al-Thwaini, 2022) and a greater risk of metabolic diseases such as ketosis and fatty liver diseases (Rico et al., 2015), which in turn can show deleterious effects on ovarian activities (Noakes et al., 2019). On the other hand, it has been shown that increased lipolysis, as well as elevated plasma non-esterified fatty acids (NEFA) concentrations, may contribute to impaired pancreatic insulin response and peripheral insulin resistance, which in turn, may promote ketogenic conditions such as ovine pregnancy toxemia (Duehlmeier et al., 2013). Insulin resistance is mediated in part by increased growth hormone (De Koster and Opsomer, 2013) and NEFA (Oliveira et al., 2016; Pires et al., 2007) and is restricted by adiponectin (De Koster et al., 2017).

Ghrelin (the hunger hormone) and adipocyte-derived cytokines (adipokine) such as leptin (satiety hormone) and adiponectin (Kubota et al., 2007), cholecystokinin, and the glucagon-like peptide 1, which also may stimulate insulin secretion are among the hormones that affect appetite and feed intake, respectively (Allen & Piantoni, 2013). It seems that adiponectin may control appetite by its effect on the hypothalamus satiety center (Kubota et al., 2007). Adiponectin is one of the most abundant plasma proteins (making up about 0.01% of all plasma proteins). The main known function of adiponectin is in fats and carbohydrate metabolism. The effects of adiponectin on the reproductive function to some extent that some researchers have discussed are common disorders associated with pregnancy (gestational diabetes mellitus, preeclampsia, preterm birth, and abnormal intrauterine growth) and the potential of adiponectin to serve as biomarkers for these disorders (Choi et al., 2020; Pheiffer et al., 2021).

The expression of adiponectin genes in bovine ovaries has been investigated in some studies suggesting the possibility that adiponectin affects bovine ovarian activity. Adiponectin can affect reproductive performance by reducing insulin resistance and metabolic consequences. The effect of adiponectin on insulin resistance is such that some human researchers introduce it as an insulin resistance marker and suggest that adiponectin > 7 mg/L in severe insulin resistance had a 97% positive predictive value for insulin receptoropathy and <5 mg/L, a 97% negative predictive value (Tabandeh et al., 2012). Ghrelin is a peptide hormone associated with controlling the appetite (hunger or appetite-stimulating hormone) consisting of 28 amino acids, in which serine 3 is modified by a fatty acid (n-octanoic acid) in the activated form. It is also associated with increased reward-seeking behaviors, including food and sex, through the stimulation of the mesolimbic dopaminergic system (Sztainert et al., 2018). Serum ghrelin concentrations are not stable in circulation and vary throughout the day. It is high before eating and decreases after nutrition. The level of ghrelin in circulation is also influenced by time and reaches its highest level throughout the night and then drops during the day. Low levels of leptin increase appetite and prepare the animal to maintain energy. After giving birth and early lactation, plasma leptin levels are low, and cows have a negative energy balance. Leptin, known to be secreted by adipocytes, is a polypeptide containing 167 amino acids with 16 kD molecular weights, carried in blood freely and or adhered to protein, and maintains a specific blood level in the plasma. Leptin's main mechanism of action is to inhibit the oscillation and expression of neuropeptide-Y to control appetite in the arcuate nucleus (Beasley et al., 2009).

Hormones such as leptin, as a satiety-inducing hormone, adiponectin (Kubota et al., 2007), and ghrelin, as a hunger-inducing hormone, can regulate the feed intake and control metabolic conditions as well as the amount of fat mobilization from the body stores, and consequently, affect the reproductive performance in the post-partum period. Leptin, ghrelin, and adiponectin, in addition to affecting food intake and metabolism, may directly affect the hypothalamic-pituitary-ovarian axis. If an association is found between adiponectin, leptin, or ghrelin in post-partum reproductive and milk production indices, it may be used to predict the mentioned indices.

This study aimed to determine the relationship between quantitative and qualitative changes in milk production, post-partum fertility indexes in cows, and changes in adiponectin, leptin, and ghrelin hormones before and after parturition.

2. Materials and Methods

Farm selection and herd management

This study was conducted on a commercial milk-producing dairy farm in the Isfahan Province, Iran, with 3800 milking cows and stable management and nutritional conditions from April to September 2018. The farm is located between 30°42′ and 34°30′ N latitude and 49°36′ and 55°32′ E longitude with an average altitude of 1600 m. The region has a sub-tropical climate. Geographical conditions of the study period were ambient air temperature of 24.57±4.56 (max-min: 6.4-40.8), rainfall of 11.56±6.31 mm, relative humidity of 24.1%±8.3%, and the average number of sunny hours per month of 313.83±48.97 h. The monthly variation in ambient temperature (30% between lowest and highest values) was higher than in relative humidity (16% between lowest and highest values). Relative humidity and ambient temperature data for the study period were obtained from the Isfahan Airport weather station. The study was approved by the Animal Care and Use Committee of the Department of Clinical Sciences of Islamic Azad University of Garmsar, Iran. On the dairy farm, cows were kept in a free-stall barn and had free access to drinking water. Cows were fed ad libitum close-up balanced total mixed ratio (TMR) cow diet twice a day, starting 3 weeks before the anticipated calving date. Cows were moved to separate covered maternity pens bedded with clean and dry straw when parturition was imminent, and they were fed the same TMR. All cows immediately after parturition were separated from the calf and were fed the same balanced TMR that met the recommended requirements of early lactating cows based on the National Research Council (2001) and milked two times a day at 05:00 and 15:00. During milking in the morning, the troughs were closed for cleaning for 30 min.

Animal selection and blood sampling

In animal selection, 45 clinically healthy Holstein dairy cows were randomly selected. They had no history of diseases in recent months. Their mean body condition score (BCS) (3.4±0.26, median=3.5), parity (1.8±1.5, median=1), age (47±19.5 months, median=39.9672 months), and weight (545±65 kg, median=510 kg) were determined and recorded at the beginning of the study. BCS was also recorded 15 days after parturition and calculated based on the previous study by Mohammad-sadegh (2019). Afterward, 15 days before and 15 days after the impending parturition, the blood samples were collected in partially evacuated plain tubes (BD, NJ, USA) from the jugular vein of each cow (6 h after the start of the morning feeding). They were immediately placed in iced water and transported to a laboratory for batch analysis before 2 h. The ambient temperature was 25°C–30°C when blood samples were collected and analyzed. The sampling time points of the 15th day before and after parturition were chosen based on Sordillo & Raphael (2013) study on the cows in the transition period. On the other hand, because metabolic syndrome and post-partum ketosis persist until about the post-partum second week, the 15th day after parturition was selected for sampling.

Laboratory measurements

In the laboratory, the samples were centrifuged at 2000×g for 15 min before the harvested serum was stored in a microtube (Easy-lock®, 1.5 mL; FL Medical Technology, Italy) at −20°C. At the beginning of animal serum tests, the samples were thawed at room temperature and spectrophotometrically analyzed using a benchtop chemistry analyzer (Erba XL-200, Germany). The concentrations of adiponectin (Bovine ADP ELISA Kit Catalog No.ABIN414074; antibodies-online GmbH Germany), leptin (Bovine LEP ELISA kit, Cusabio Biotech Co. China), ghrelin (Bovine GHR ELISA kit, MyBioSource Co. USA), glucose (Hexakinase method; Glucose commercial Kit; Parsazmun, Iran), insulin (Bovine INS commercial Kit, Korean Biotech Co. China), βHBA (BHB dehydrogenase method, Ranaut, Randox Ltd, Antrim, UK).), urea (commercial Kit; Parsazmun Co., IRAN), and creatinine (commercial Kit; Parsazmun Co., Iran) were measured in the serum samples according to the catalog based ELISA method. Serum adiponectin concentrations were measured by a competitive ELISA method. The microtiter plate contained in the kit was pre-coated with a specific ADP antibody. The standard solution of samples for which we intend to measure adiponectin concentrations was added to the wells along with the ADP biotin conjugate. The antibody binds less with the ADP biotin conjugate in samples with higher adiponectin levels. After the plate washing, the HPR was added to all wells and fixed with a stop-dye solution, and the ELISA reader measured the color intensity. The measurable concentrations of adiponectin in the used kit range from 3.12 to 50 μg/mL. The postpartum cows with serum BHB concentrations of more than 1.1 mmol/L were considered ketotic cows based on Constable et al. (2017). Adiponectin, leptin, and ghrelin concentration and the first service conception rate were compared between the ketotic and normal cows.

Study hypotheses and variables

Serum adiponectin, leptin, and ghrelin concentrations were assumed as independent variables in the hypothesis. They can affect reproductive indexes, milk production levels, and milk constitution. Therefore, levels of post-partum fat, protein, and milk production, the rate of pregnancy following the first artificial insemination (AI), days to the first service (DFS), parturition to pregnancy intervals (days open), and services per conception were considered dependent variables in this hypothesis. On the contrary, parturition, the length of the dry period, the length of pregnancy, and calf weight were assumed as independent (predictor) variables in the hypothesis that they could affect the concentrations of adiponectin, leptin, and ghrelin. Thus, the mentioned hormones were considered dependent (criteria) variables in the last hypothesis. BCS, βHBA, parity, age, urea, creatinine, glucose, and insulin at 15 before and 15 days after the expected parturition were considered independent variables.

Statistical analysis

Serum concentrations of adiponectin, leptin, ghrelin, glucose, insulin, urea, and creatinine were presented as Mean±SD in data with a normal distribution and as medians and the interquartile in data with non-normal distribution.

The quantitative data were first analyzed with the Shapiro-Wilk and the Kolmogorov-Smirnov tests to evaluate the normality of the distribution. After that, the data were compared between the two groups (cows before and after parturition, or pregnant and non-pregnant following the first AI) with the Wilcoxon (in pairs data) or Mann-Whitney U test in the non-normally distributed data or with the student t-test in a normally distributed data.

The association between the quantitative data was analyzed with the bivariate Pearson correlation test. Finally, the Cox regression statistical model was used to evaluate the effects of covariates on the effects of adiponectin, leptin, and ghrelin on the calving to conception intervals (days open). The logistic regression statistical model was also utilized to predict the constancy of adiponectin, leptin, and ghrelin serum concentrations on the first service conception rate, together with many other covariates.

All statistical analyses were performed using software programs MedCalc, version 13.3.3 (MedCalc Software, Ostend, Belgium, 2015) and SPSS software, version 24 (IBM Co., New York 10504-1722, USA). $P<0.05$ was considered statistically significant.

3. Results

The findings showed that the assumption of normality in the distribution of quantitative data was accepted in adiponectin, insulin, leptin, and ghrelin before and after parturition, and urea only after parturition, length of last pregnancy, and parturition to pregnancy intervals ($P>0.05$).

Adiponectin (14.6±3.2 and 16.65±2.2 μg/mL, P=0.002), ghrelin (155±0.78 and 21.3±0.8 pg/mL, $P<0.001$) and leptin concentrations (8.02±0.55 and 6.05±0.54 ng/mL, $P<0.001$), before and after parturition (respectively) were measured, and revealed some differences (Table 1). Insulin, glucose, urea, and creatinine concentrations before parturition also showed a difference in comparison with their concentrations after parturition ($P<0.001$) (Table 1). The ratio of adiponectin to leptin (1.8306±0.44 and 2.7608±0.33, $P<0.001$) and leptin to ghrelin (0.51812±0.025 and 0.28340±0.02, $P<0.001$) before and after parturition were also analyzed, and showed some differences, too (Figure 1).

In the samples collected on the 15th day before the parturition, concentrations of adiponectin showed no association between leptin (r=-0.019, P=0.9) and ghrelin (r=0.023, P=0.88) and had a negative association with the BCS (r=-0.73, $P<0.001$). In pre-partum cows with BCS≤3, the average serum adiponectin concentration (15.4±3 μm/mL) was more than in cows with BCS>3 (11±1.2 μm/mL) ($P<0.001$) (Figure 2). There was no relationship between the concentration of adiponectin 15 days before the parturition and variables of age, parity, the length of the dry period, and the calf weight at caving ($P>0.05$). Leptin concentrations re-

Table 1. Comparing adiponectin, leptin, and ghrelin concentrations before and after parturition (P)

Criteria	Periparturient Time at Sampling	Mean±SD	95% Confidence Interval for Mean		Median	95% CI for the Median		Min	Max	P
			Lower	Upper		Lower	Upper			
Adiponectin (μg/mL)	Before	14.60±3.3^{a†}	13.6	15.575	15.43	12.717	16.582	8.89	20.60	0.002
	After	16.65±2.2^{b}	16.0	17.296	16.78	16.185	17.184	12.50	21.22	
Leptin (ng/mL)	Before	8.02±0.55^{a}	7.9	8.18	8.0	7.800	8.154	7.0	9.4	0.001
	After	6.05±0.54^{b}	5.9	6.21	6.0	5.800	6.154	5.0	7.2	
Ghrelin (pg/mL)	Before	15.5±0.78^{a}	15.2	15.71	15.7	15.192	15.900	14.0	17.0	0.001
	After	21.3±0.8^{b}	21.1	21.57	21.5	21.000	21.754	19.7	22.9	
Insulin (ng/mL)	Before	5.09±0.42^{a}	4.96	5.22	5.1	4.946	5.200	4.2	6.0	0.001
	After	4.79±0.45^{b}	4.7	4.93	4.8	4.600	4.954	3.9	5.9	
Glucose (mg/dL)	Before	53.15±3.1	52.2	54.09	53.2^{a}	52.384	54.854	44.60	58.50	0.001
	After	48.36±3.7	47.26	49.5	50.0^{b}	47.800	50.300	40.00	53.70	
Urea (mg/dL)	Before	15.7±1.6	15.2	16.2	15.4^{a}	14.892	16.054	13.0	19.0	0.001
	After	15.1±1.5	14.7	15.6	15.0^{b}	14.492	15.408	12.2	18.5	
Creatinine (mg/dL)	Before	1.22±0.16	1.18	1.27	1.16^{a}	1.140	1.185	1.09	2.00	0.001
	After	1.25±0.16	1.2	1.29	1.19^{b}	1.175	1.211	1.03	2.04	

Standard deviation, $^{a, b}$Significantly different group (P<0.05).

vealed a relationship with ghrelin (r=0.69, P<0.001), age (r=0.464, P=0.001), parity (r=0.48, P=0.001), insulin concentrations (r=0.89, P<0.001), and the length of dry period (r=-0.445, P=0.005).

Ghrelin concentration had an association with the age of cows (r=0.435, P=0.003), parity (r=0.442, P=0.002), and insulin (r=0.76, P<0.0001).

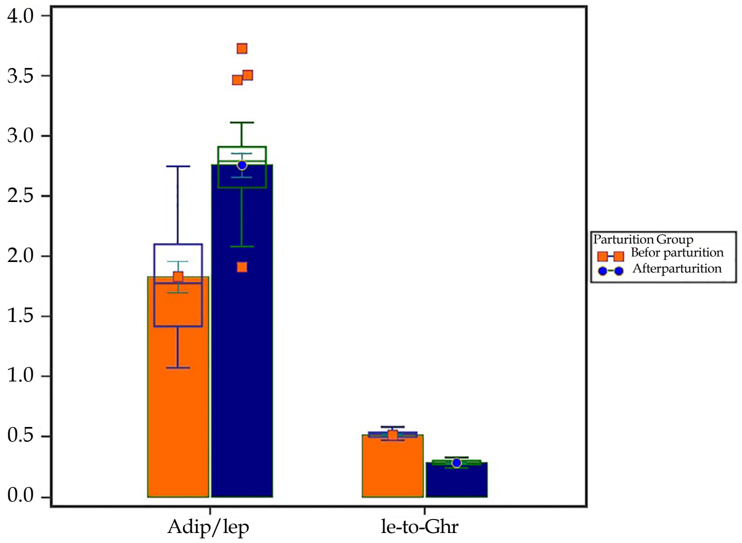

Figure 1. Adiponectin to leptin (P<0.001), and leptin to ghrelin (P<0.001) ratio before and after parturition

Table 2. Comparing some biochemical parameters between the pregnant (n=15) and non-pregnant (n=30) cows following the first AI

Criteria		Pregnancy First AI	Median	Mean±SD	Mean±SE	95% CI for Mean Lower	95% CI for Mean Upper	Min	Max	P
Adiponectin (µg/mL)	Before P	No	14.7	14.5±3.3[a†]	14.5±0.6[a†]	13.3	15.8	8.9	20.6	0.830
		Yes	16.2	14.8±3.3[a]	14.8±0.8[a]	12.9	16.6	9.5	19.2	
	After P	No	16.4	16.4±2.1[a]	16.4±0.4[a]	15.6	17.2	12.5	20.2	0.310
		Yes	17.6	17.1±2.3[a]	17.1±0.6[a]	15.9	18.4	13	21.2	
Leptin (ng/mL)	Before P	No	7.8	7.8±0.4[a]	7.8±0.1[a]	7.7	8	7	8.7	0.004
		Yes	8.6	8.4±0.6[b]	8.4±0.2[b]	8.1	8.7	7	9.4	
	After P	No	5.9	5.9±0.5[a]	5.9±0.1[a]	5.7	6.1	5	7	0.015
		Yes	6.5	6.3±0.6[b]	6.3±0.2[b]	6	6.7	5	7.2	
Ghrelin (pg/mL)	Before P	No	15.3	15.2±0.7[a]	15.2±0.1[a]	14.9	15.5	14	16.5	0.001
		Yes	16	16±0.7[b]	16±0.2[b]	15.6	16.4	14.4	17	
	After P	No	21	21.1±0.7[a]	21.1±0.1[a]	20.8	21.4	19.7	22.3	0.007
		Yes	21.9	21.8±0.8[b]	21.8±0.2[b]	21.4	22.2	20.1	22.9	
Insulin (ng/mL)	Before P	No	5	5±0.3[a]	5±0.1[a]	4.8	5.1	4.2	5.6	0.011
		Yes	5.4	5.3±0.5[b]	5.3±0.1[b]	5.1	5.6	4.4	6	
	After P	No	4.7	4.7±0.4[a]	4.7±0.1[a]	4.5	4.8	3.9	5.5	0.022
		Yes	5	5±0.5[b]	5±0.1[b]	4.8	5.3	4	5.9	
Glucose (mg/dL)	Before P	No	53.7[a]	53.5±3.2	53.5±0.6	52.3	54.7	44.6	58.5	0.270
		Yes	52.2[a]	52.4±2.9	52.4±0.7	50.9	54	46.8	56	
	After P	No	50.1[a]	49±3.6	49±0.6	47.7	50.4	40	53.7	0.04
		Yes	46.8[b]	47±3.6	47±0.9	45	49	41.5	52.2	
Urea (mg/dL)	Before P	No	15.3[a]	15.7±1.6	15.7±0.3	15	16.3	13	19	0.8
		Yes	1.4[a]	15.7±1.6	15.7±0.4	14.8	16.6	14	18.8	
	After P	No	15[a]	15.1±1.5	15.1±0.3	14.6	15.7	12.2	18.5	0.9
		Yes	15[a]	15.1±1.6	15.1±0.4	14.2	16	13	18.3	
Creatinine (mg/dL)	Before P	No	1.2[a]	1.2±0.2	1.2±0.03	1.2	1.3	1.1	2	0.9
		Yes	1.2[a]	1.2±0.1	1.2±0.03	1.1	1.3	1.1	1.42	
	After P	No	1.2[a]	1.3±0.2	1.3±0.03	1.2	1.3	1.1	2.04	0.8
		Yes	1.2[a]	1.2±0.1	1.2±0.03	1.2	1.3	1	1.42	

P: Parturition. [†, a, b]Significantly different group (P<0.05).

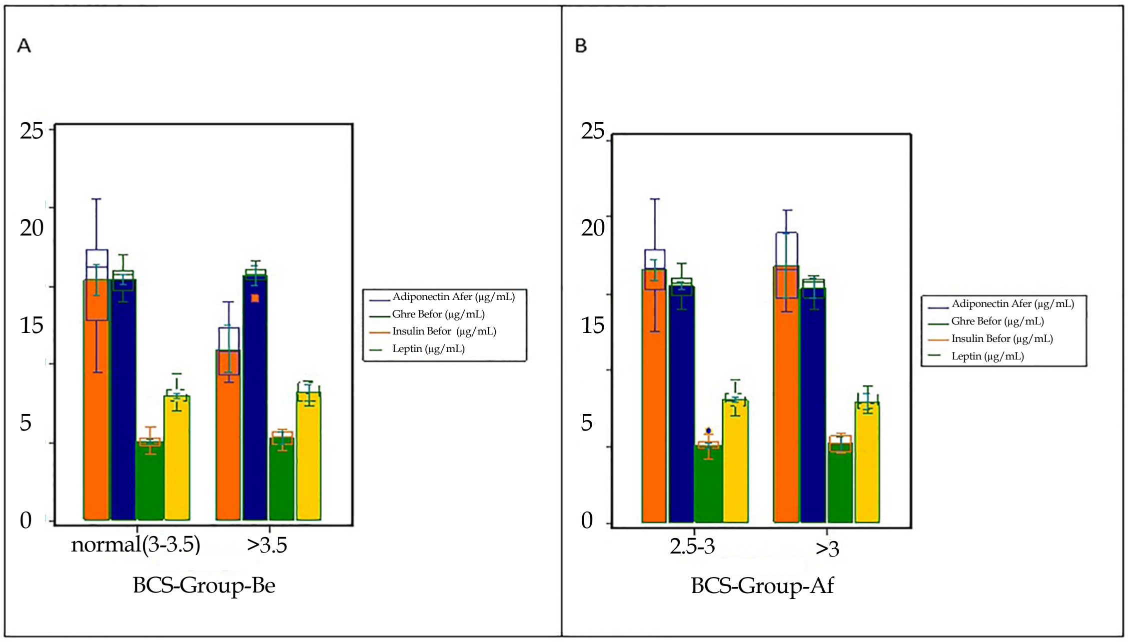

Figure 2. Comparing concentrations of adiponectin, ghrelin, insulin, and leptin before and after parturition between the different BCS groups

In the samples collected on the 15th day after parturition, the concentrations of adiponectin showed a statistical association with leptin (r=0.46, P=0.001), ghrelin (r=0.35, P=0.019), and insulin (r=0.344, P=0.02). There was a relationship between the serum concentration of adiponectin and the calves' weight at parturition (r=0.336, P=0.024). No association was found between the serum concentration of adiponectin and age, parity, the length of the dry period, and post-partum BCS of cows (P>0.05). In post-partum cows with the BCS≤3, the average serum adiponectin concentration (16.5±2 µm/mL) was not statistically more than in cows with the BCS>3 (16.8±2.5 µm/mL) (P<0.001) (Figure 2). Leptin concentrations revealed a statistical relationship with ghrelin (r=0.55, P=0.000), age (r=0.34, P=0.023), parity (r=0.36, P=0.015), the length of dry period (r=0.32, P=0.049), and insulin (r=0.55, P<0.001). Ghrelin concentrations had an association with the age of the cows (r=0.38, P=0.01), parity (r=0.38, P=0.01), and insulin (r=0.67, P=0.001). A comparison of serum biochemical parameters between the pregnant and non-pregnant cows following the first AI showed no difference in adiponectin concentration; however, pre- and post-partum serum concentrations of leptin, ghrelin, and insulin were higher in pregnant cows following the first AI. Nevertheless, only post-partum serum glucose concentration was lower in pregnant cows at the first AI (Table 2).

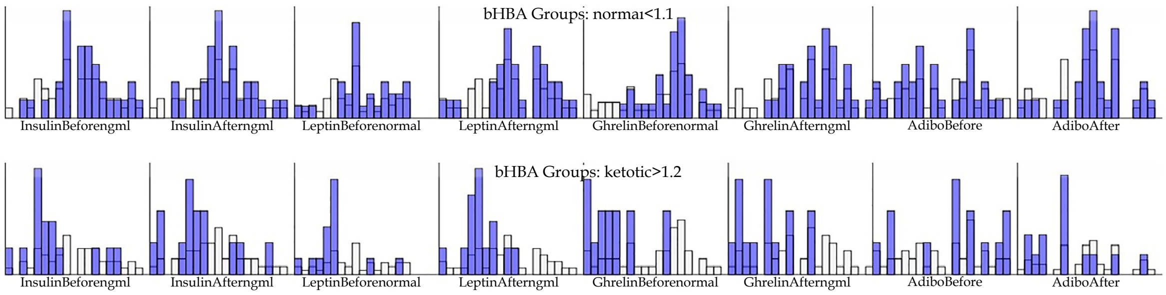

Figure 3. Comparing biochemical parameters with a statistical difference between the normal (low βhba) and ketotic (high βhba) cows post-partum adiponectin concentration, and pre and post-partum leptin, ghrelin, and insulin concentrations showed a lesser level in ketotic cows. P<0.05.

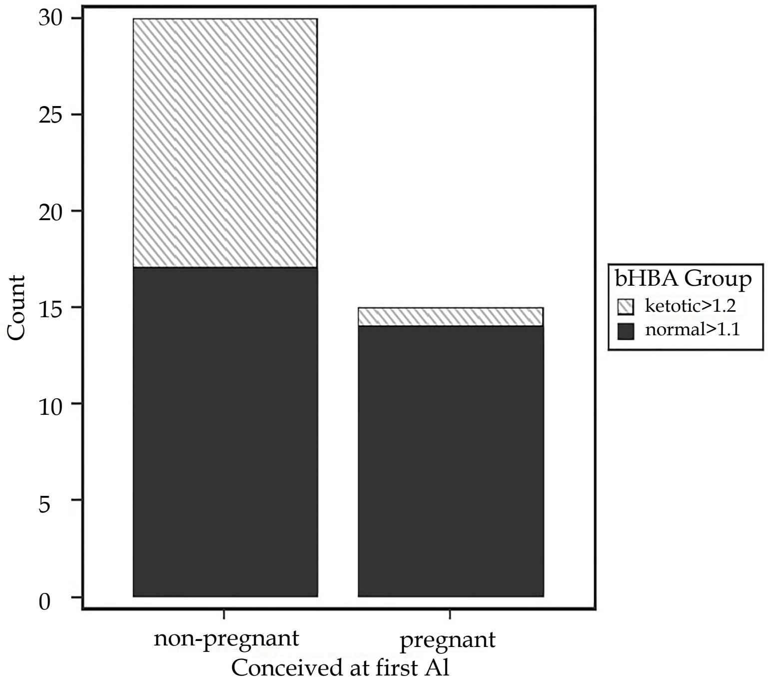

Figure 4. Comparing between the absolute number of pregnant and non-pregnant cows following the first AI in ketotic (high βHBA) and normal (βHBA) cows (P=0.011)

A comparison of biochemical parameters between the ketotic (high βHBA) and non-ketotic (low βHBA) cows revealed that the pre-partum adiponectin concentration and pre- and post-partum glucose, urea, and creatinine concentrations had no differences (P>0.05). However, post-partum adiponectin concentration and pre- and post-partum leptin, ghrelin, and insulin concentrations showed lower levels in ketotic cows (Figure 3) (P<0.05).

Of 15 conceived cows following the first AI, 14 cows were non-ketotic, and only one cow suffered from ketosis (Figure 4). However, from 30 non-conceived cows following the first AI, 17 were non-ketotic, and 13 suffered from ketosis (P=0.011).

The adiponectin concentration showed a negative association with the milk production levels (r=-0.5, P=0.00) (no association with milk fat or protein) and service per conception (r=-0.4, P=0.009) (Figure 5).

Leptin concentration revealed negative correlations with the milk fat levels (r=-0.37, P=0.013) (no association with milk production or milk protein levels), the period of the first AI to pregnancy (r=-0.56, P<0.0001), and days open (r=-0.04, P=0.003) (Figure 5).

Ghrelin concentrations had a positive association with the milk production levels (r=0.034, P=0.02) (no association with milk protein) and negative correlations with milk fat levels (r=-0.32, P=0.03), the period of the first AI to pregnancy (r=-0.57, P=0.000) and days open (r=-0.43, P<0.0001) (Figure 5).

Insulin concentration showed negative correlations with the period of the first AI to pregnancy (r=-0.52, P<0.0001) and days open (r=-0.41, P=0.005). It also revealed a negative association with glucose concentrations (r=-0.32, P=0.031).

Finally, the Cox regression statistical model showed that the effects of adiponectin, leptin, and ghrelin on the length of parturition to pregnancy (days open) were not constant against the other metabolic-biochemical, or managemental covariates (BCS, age, the length of the dry period, calf weight, milk production, milk protein and fat, and post-partum reproductive disorders) (P>0.05). Also, the logistic regression analysis showed that the effects of adiponectin, leptin, and ghrelin on the pregnancy at the first AI were not stable against the other metabolic-biochemical or managemental covariates (P>0.05).

4. Discussion

Based on the present study's findings, serum adiponectin and ghrelin concentrations increased, and serum leptin levels were reduced after parturition. Serum adiponectin concentrations have been studied by many research-

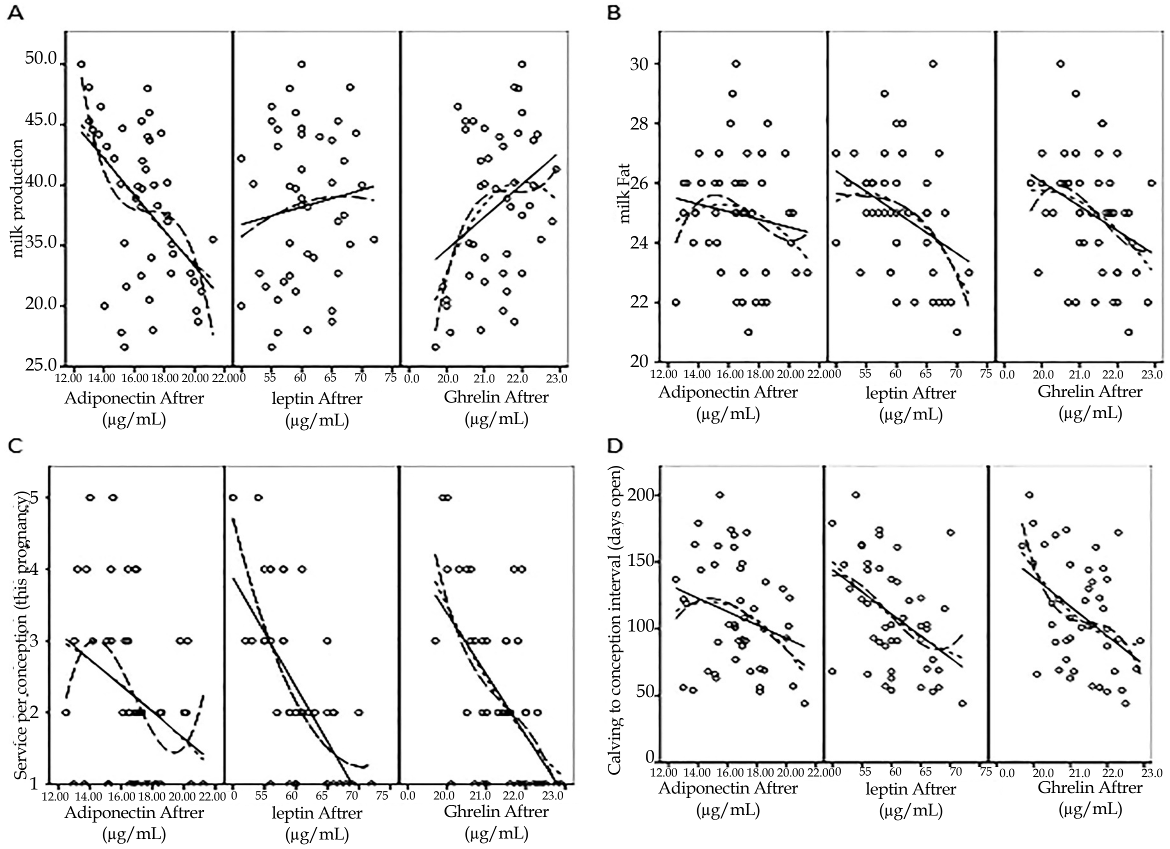

Figure 5. Visualization of scatter plot of linear fit model and the effect of leverage for detecting the relationship between milk production levels with serum concentrations of A) Adiponectin (r=-0.5, P=0.00), leptin (r=0.12, P=0.4), and Ghrelin (r=0.034, P=0.02), B) Milk fat levels with serum concentrations adiponectin (r=0.12, P=0.4), Ghrelin (r=-0.32, P=0.03), Leptin (r=0.37, P=0.013), C) Service per conception with serum concentration of adiponectin (r=-0.32, P=0.03), leptin (P>0.05), and ghrelin (P>0.05), D) Calving to conception interval (days open) with adiponectin (P>0.05), leptin (r=-0.04, P=0.003) and ghrelin (r=-0.43, P=0.000).

ers and showed a decrease at the end of the dry period in dairy cows (De Koster et al., 2017), reached a nadir at calving, and increased throughout lactation (Ohtani et al., 2012). De Koster et al. (2017) enrolled only 10 cows, and Singh et al. (2014) used 50 multiparous cows in their research to study adiponectin concentrations. In the present study, the same number of cows was enrolled. Considering the deduction of the means of pre- and post-partum adiponectin, their standard deviation, and the ratio of the samples were used to calculate the sample size based on the MedCalc software, the suggested sample size was 40 cows in pre- and post-partum cows (type I error=0.05, type II error=0.10 and the power of the test=90%).

Higher post-partum adiponectin concentrations regulate insulin resistance and its metabolic postponements. In rodents and primates, insulin resistance develops during pregnancy and decreases after parturition due to serum adiponectin changes. However, insulin resistance is maintained in dairy cows via decreased serum adiponectin concentrations in early lactation to favor mammary glucose uptake. This reduction occurred without any change in the medium molecular weight distribution of plasma adiponectin or adiponectin mRNA abundance in the white adipose tissue; however, it occurred via a reduction in high molecular weight adiponectin (Giesy et al., 2012).

Based on the findings of the current investigation on the 15th day before the anticipated calving time, the lack of an association of the adiponectin concentration with the leptin, ghrelin, and insulin levels, and a strong correlation of leptin and ghrelin with the insulin concentrations may emphasize the importance of leptin and ghrelin in energy and glucose metabolism. On the other hand, the lack of association of leptin and ghrelin with the BCS and a strong negative correlation (r=-0.73, P<0.001) between adiponectin and the BCS show the importance of adiponectin in fat mobilization and or metabolism. In our study, serum adiponectin concentrations were more in pre-partum cows with BCS≤3.5, and serum adiponectin concentrations were higher than in cows with BCS>3.5.

In agreement with the present research, serum adiponectin concentration during the dry period in some studies was negatively associated with the BCS (De Koster et al., 2017). It has been shown that the increased serum adiponectin might be involved in energy metabolism just around parturition (Ohtani et al., 2012). The findings of the current research 15 days after the parturition, similar to the study of Giesy et al. (2012), i.e. the higher levels of adiponectin and ghrelin concentrations and lower amounts of leptin concentration may be a reaction to the increased demand for feed intake and a reduced in insulin resistance rate.

In the present study, the pre-partum adiponectin concentration and pre- and post-partum glucose, urea, and creatinine concentrations had no difference between the healthy and ketotic cows. However, post-partum adiponectin concentration and pre and post-partum leptin, ghrelin, and insulin concentrations showed lower levels in ketotic cows.

In agreement with our study, Akgul et al. (2018) found that adiponectin had a lower level in dairy cows with both clinical and subclinical ketosis on day 7 post-partum compared to the healthy cows (Akgul et al., 2018).

Our study found a very low first service conception rate (7% rather than 45%) in cows suffering from ketosis. The associations among the concentrations of adiponectin, leptin, ghrelin, and insulin greatly emphasize their activities in the field of energy metabolism in early lactation. Consistent with our study, serum adiponectin concentrations are positively associated with insulin responsiveness in many research studies (De Koster et al., 2017).

Contrary to the current study, the findings of a research on young female sheep showed that the leptin concentration had a negative association with age and revealed a positive correlation with the live weight at the first estrus and the proportion of females that attained puberty, and with the fertility rate (Rosales Nieto et al., 2015).

In the present investigation, the lack of an association of adiponectin with the days to the first service, or days open, a negative correlation with the service per conception, and the presence of a relationship of leptin and ghrelin on the period of the first AI to pregnancy, and days open may depict the importance of leptin and ghrelin in ovulation, fertilization, implantation, and or early embryonic developments. Furthermore, none of the studied hormones in the current study correlated with days to the first service, a post-partum period in which the ovaries become active. On the other hand, the comparison of serum biochemical parameters between the pregnant and non-pregnant cows following the first AI showed no difference in adiponectin concentration in the present study. However, pre- and post-partum serum concentrations of leptin, ghrelin, and insulin were higher in conceived cows following the first AI. Nevertheless, only post-partum serum glucose concentration was lower in pregnant cows on the first AI. Based on the findings of Oliveira et al. (2016), the increased level of insulin in pregnant cows following the first AI in our study raises the possibility of insulin resistance. However, the decreased concentration of glucose in pregnant cows following the first AI is a questionable finding in our study. In this study, we may encounter a state in which insulin levels increase via ways other than insulin resistance, probably through the other hormones. Concerning the fact that insulin resistance is characterized by a reduced action of insulin even though hyperinsulinemia and insulin resistance is restricted by adiponectin (De Koster et al., 2017), a negative association between the serum adiponectin and insulin concentrations has been assumed in the present study. However, the current study's findings show a positive correlation between the serum adiponectin and insulin concentrations 15 days after parturition. Adiponectin can affect female animal reproduction by its effects on the pituitary gland, LH secretion, and theca cells (Lu et al., 2008). In a study in bovine healthy follicles, follicular growth was correlated with an increase in the gene expression of adiponectin and its receptors in the granulosa and cumulus-oocyte complex. In contrast, adiponectin expression decreases in theca cells across the follicular development. Regression of the corpus luteum was related to increased expression of adiponectin and its receptors (Tabandeh et al., 2012). Contrary to the lack of an association between adiponectin and the days to the first service, or days open in the present study, there are accumulating pieces of evidence for the direct effects of adipokine on the GnRH and LH production and function, steroidogenesis, the late stages of folliculogenesis and on the development of a functional placenta (Dos Santos et al., 2012).

The lack of association with adiponectin on the days to first service (DFS), the period of the first AI to pregnancy, and days open in the current paper may indicate no effect of adiponectin on post-partum ovarian activity. In a study, adiponectin concentrations in cows with the commencement of luteal activity greater than 45 days decreased more than those with the commencement of luteal activity 45 days or less after week 3 post-partum. Serum adiponectin concentrations at week 7 post-partum were lower in the delayed commencement of the luteal activity. Serum adiponectin concentrations decreased gradually by week 3 post-partum in cows with normal luteal activities and then increased, whereas in an-ovulatory cows and delayed first ovulation, they were decreased after week 3 post-partum. Moreover, serum adiponectin concentrations in normal luteal activity were more than in an-ovulatory cows at weeks 5 and 7 post-partum. It is revealed that the expression of ADIPOQ and ADIPOR2 (receptors of adiponectin) genes on chromosome 3 are the only genes showing a significant mRNA overexpression in infertile cows at the stage of fat mobilization, and an increase of non-esterified fatty acid (NEFA) levels (Kafi et al., 2015). Furthermore, leptin stimulates gonadotropin secretion at the hypothalamus and pituitary levels to modulate ovarian steroidogenesis, embryonic implantation, and placental functions (Guerre-Millo, 2008). Because ghrelin reveals a direct regulatory effect on GnRH neurons and modulates the surge of GnRH in an ovarian cycle (Prajapati et al., 2018), it is expected in the present study that the ovulation risk and the conception rate increase and days open decrease in the cases with higher concentrations of ghrelin. On the other hand, it is reported that the release of GnRH and the function of the reproductive endocrine system are determined by the balance of positive signals (IGF1, leptin) and negative signals (Ghrelin) at GnRH neurons (D'Occhio et al., 2019).

However, the findings of the present investigation showed that the serum leptin concentrations had a negative correlation with the period of the first AI to pregnancy (r=-0.56, P=0.000) and calving to conception interval (days open) (r=-0.04, P=0.003) in post-partum cows. Besides, in agreement with the findings of D'Occhio et al. (2019), ghrelin showed negative associations with the period of the first AI to pregnancy (r=-0.57, P=0.000) and days open (r=-0.43, P=0.000) in our study.

In the present study, milk production levels were positively associated with ghrelin and negatively correlated with adiponectin concentration. On the other hand, milk fat levels were negatively related to leptin and ghrelin concentration. In agreement with our findings, the milk fat levels showed a negative correlation with serum ghrelin and leptin concentrations in some other studies (Elis et al., 2013). Regarding results that leptin single nucleotide polymorphisms were associated with fertility (UASMS1, UASMS2, A1457G, A59V) and milk production (A59V) genes (Clempson et al., 2011) we assumed that milk production or reproductive indexes were related to serum leptin concentrations.

Finally, no constant effect of the adiponectin, leptin, and ghrelin on the calving to conception interval and pregnancy in the first insemination showed a lower effect of the hormones on reproduction indexes than the effects of other covariates. On the other hand, the impact of adiponectin on milk production levels probably showed that the strongest activity of the hormone is the regulation of metabolism. More studies are needed to investigate their effects on reproductive performance in field conditions. It is important to notice that the sample size of the present study does not seem suitable for evaluating the constancy of the hormones by the logistic and Cox survival analysis.

5. Conclusion

In conclusion, this study showed that the serum concentrations of adiponectin and ghrelin increased, and the serum levels of leptin decreased after parturition. However, these patterns of changes could not have a long-lasting effect on the reproductive and milk production indexes along with the studied covariances and confounding variables. Therefore, the possibility of predicting post-partum reproductive and milk production indexes via the concentrations of adiponectin, leptin, and ghrelin tends is not encouraging. Other studies with more samples are needed to better understand the effect of the measured hormones on reproductive and milk production indexes.

Ethical Considerations

Compliance with ethical guidelines

The study plan and ethical considerations were approved by the Ethics and Animal Care and Use Committee of the Department of Clinical Sciences, Faculty of Veterinary Medicine, Garmsar Branch, Islamic Azad University. The animals included in the study were in a milk producing commercial dairy farm and did not undergo any manipulation or injection. On the other hand, the research planning was a observational study and did not need to any ethical consideration.

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Authors' contributions

Conceptualization, methodology, investigation, farm animal sampling and efforts, laboratory coordination: Mohsen Ketabi; Project administration, data curation and analysis, writing the original draft, review & editing: Majid Mohammad-Sadegh.

Conflict of interest

The authors declared no conflict of interest.

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مقاله پژوهشی

غلظت سرمی ادیپونکتین، لپتین و گرلین قبل و بعد از زایمان و توانایی آنها در پیش بینی عملکرد تولید مثلی و شاخص های تولید شیر در گاوهای شیری هلشتاین

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چکیده

زمینه مطالعه: ادیپونکتین، لپتین و گرلین عوامل تنظیم کننده متابولیسم هستند که ممکن است بر تولید شیر و عملکرد تولید مثلی تأثیر بگذارند.

هدف: هدف از این مطالعه بررسی توانایی غلظت سرمی ادیپونکتین، لپتین و گرلین در پیش بینی شاخص‌های تولیدمثلی و تولید شیر گاوهای هلشتاین در دوره پس از زایمان بود.

روش کار: در این مقاله چهل و پنج راس گاو شیری هلشتاین از نظر بالینی سالم و آبستن در نزدیکی زایش به طور تصادفی انتخاب شدند. در پانزدهمین روز قبل و بعد از زایمان از ورید گردن گاوها نمونه خون گرفته شد و غلظت سرمی ادیپونکتین، لپتین و گرلین و برخی دیگر از پارامترهای بیوشیمیایی متابولیک اندازه‌گیری شد و حیوانات در سه ماه متوالی از نظر تولید شیر و پارامترهای تولیدمثلی پایش شدند.

نتایج: یافته ها نشان داد که تفاوت معنی داری در غلظت ادیپونکتین بین گاوهای باردار و غیر آبستن پس از اولین تلقیح یافت نشد. با این حال، غلظت سرمی لپتین، گرلین و انسولین قبل و بعد از زایمان در گاوهای آبستن پس از اولین تلقیح بالاتر بود (P>۰/۰۵). بین غلظت سرمی ادیپونکتین پس از زایمان و تعداد تلقیح به ازای آبستنی و میانگین سطح تولید شیر همبستگی منفی معنی‌داری مشاهده شد (P>۰/۰۵). با این حال، همبستگی ادیپونکتین به دلیل سایه سایر متغیرهای مورد مطالعه نتوانست تأثیر پایداری بر شاخص‌های تولید مثل و تولید شیر داشته باشد.

نتیجه‌گیری نهایی: از این تحقیق نتیجه گیری شد که افزایش ادیپونکتین و گرلین سرم و کاهش غلظت لپتین پس از زایمان در شرایط این مطالعه قادر به پیش بینی شاخص های تولید مثلی و تولید شیر نبود.

کلیدواژه‌ها: ادیپونکتین؛ گرلین؛ لپتین؛ شاخص های تولید شیر؛ شاخص های تولید مثل

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