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The relationship of leptin and thyroid hormones with milk yield and some fertility parameters in Simmental dairy cows during transition period

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Abstract: In this study, transition period cows were used to test the hypothesis that serum levels of leptin and thyroid hormones would correlate the production efficiency such as milk yield, body condition score, and fertility in dairy cows raised under the same controlled conditions. The study comprised 37 Simmental cows, which were 3 years old and between 257–265 days of gestation. Serum levels of leptin, T3, T4, FT3, and FT4 hormones were measured in blood samples obtained from the animals, which were in the period of 3 weeks before and 3 weeks after delivery. Prepartum serum leptin levels were significantly ($p < 0.05$) lower in cows having increased milk production compared to the first lactation peak value. Also, postpartum serum FT3 levels were significantly ($p < 0.05$) lower in cows having milk yield of ≥ 30 kg. Leptin levels were found to be inversely related with milk production and fertility. FT3 levels exhibited a negative correlation with milk production and similarly T3 levels showed an inverse correlation with body condition score. The present study proposed that routine monitoring of leptin and thyroid hormones would be useful for the reproductive management of cows with breeding problems.

Key words: Leptin, thyroid hormones, transition period, milk yield, fertility parameters, dairy cows

1. Introduction

In cows, the transition period is theoretically defined as 3 weeks [1,2,3,4] or 4 weeks [5] before and after calving, while the period from 1 week before to 2 weeks after delivery is specified as the most important period in practice [2]. In this period, the incidence of many diseases such as hypocalcemia, hypomagnesemia, retentio secundinarum, metritis, ketosis, left abomasum displacement, lameness, downer cow syndrome, and clinical mastitis increases [3,5].

During the period of transition, sudden changes occur in metabolic and immune functions of cows. Some of these changes are associated with increased energy needs due to fetus and lactogenesis [3]. Although the need for energy increases, dry matter intake decreases by 10%–30% due to physical, behavioural, metabolic, and hormonal changes in the period prior to delivery [3,4]. The transition period in cows has attracted the attention of researchers, and studies have been previously conducted to investigate many parameters such as urea, albumin, globulin, total protein, serum bilirubin, glutamate dehydrogenase, gamma glutamyl transferase, aspartate aminotransferase, haptoglobulin, cortisol, triiodothyronine (T3), thyroxine (T4), calcium, and magnesium [4,6,7,8,9].

Some hormones control the metabolic adaptation in the transition period. Leptin plays an important role during this adaptation by coordinating feed intake, energy expenditure, and nutrient use in tissues. Leptin has an autocrine / paracrine effect on the inhibition of lipogenesis and stimulation of lipolysis [10,11]. As a protein hormone, leptin is synthesized almost exclusively by adipocytes. The amount of secretion is proportional to the degree of fat in the body. It reduces voluntary feed consumption with its effect on the central nervous system. In addition, it creates peripheral insulin resistance in ruminants in the periparturient period [11,12,13]. The high leptin concentration observed prior to calving in multiparous cows is a strong indicator of the delay in the first postpartum ovulation. Prolonged first service period and conception interval have also been associated with high leptin. The body condition score (BCS), closely related to leptin, can be used as an indicator of adipose tissues that will later be mobilized to support lactation. A decrease in dry matter intake due to increased leptin concentration may also result in greater BCS loss [13].

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The thyroid gland also plays a role in the transitional metabolic adaptation. Thyroid hormones, especially T3, play an important role in the regulation of energy metabolism [9]. Thyroid hormones affect the initiation of ovarian activities. The lack of ovarian activity in animals with low T3 and T4 concentrations can be given as an example of its reproductive importance. Similarly, in animals with T3 concentration below 1.4 nM, low estradiol concentration and a decrease in oestrus symptoms accompany [14]. When there is mobilization of body reserves for high milk production in cows in the peripartal period and especially in the early lactation period, there is a decrease in thyroid hormone levels. At the beginning of lactation, negative energy balance, lipomobilization, and hypothyroidism pose a risk of carbohydrate and lipid metabolism diseases such as ketosis and fatty liver syndrome, especially due to disruption in energy metabolism and oxidation processes in liver cells of dairy cows. T3 and T4 levels are considered as indicators of adaptation to negative energy balance until energy balance is achieved [9].

The use of blood parameters to assess health status on herd basis during the transition period has been an issue that has been studied for years. Making these follow-ups is also important for economic issues such as milk yield and reproductive performance. However, the problems of the transition period are still not fully resolved. Thus, this study aimed at investigating the relationship of serum leptin, T3, T4, FT3, and FT4 levels of transitional cows with milk yield, BCS, and some fertility parameters.

2. Materials and methods

2.1. Animals

The study was conducted on a large dairy farm in Yozgat province of Turkey. While the animals in the enterprise were fed with a sufficient amount of roughage and concentrate feed, the barn type of the enterprise was semi-open. The cows that parturiated were given intramuscular PGF2α, 3 times, on the 14th, 21st, and 28th days postpartum and intrauterine oxytetracycline washing was applied once in the 30–32 days period. Oestrus activity was determined by observation and confirmed by rectal and ultrasonographic examinations.

The presented study was conducted with the permission (Date: 29.12.2020, No: E.8420) of the Animal Experiments Local Ethics Committee of Kırıkkale University. The study consisted of 37 Simmental cows, which were 35–38 months old, with the previous first parturition and milk yield records and were between 257–265 days of gestation.

2.2. Blood samples, body condition scores, and milk yields

Blood samples were collected twice from the v. jugularis of each animal. First samples were taken 257–265 days during pregnancy and the second blood samples were also taken 15–18 days postpartum. The collected blood samples were centrifuged (CN 180, Nuve, TR) at 3000 rpm for 10 min, and their serum was removed. Serums were aliquoted into 1.5 mL snap cap tubes (Eppendorf, Germany) and stored at –20 °C until laboratory analysis.

BCS was determined and recorded using a 5 - point BCS system [15]. The same veterinarian carried out the valuation throughout the study. The milk yields of all the animals in the study were recorded on the 8th week postpartum.

2.3. ELISA analyses

The quantification of leptin, T3, T4, FT3, and FT4 in serum samples were performed by ELISA method. Analyses were carried out by adherence to the protocols recommended by commercial ELISA kits (Cusabio USA). Using the double-antibody sandwich-based kits, the measurements of the standard solutions were made at five different concentrations.

Standard and streptavidin HRP samples were added to the standard wells of the ELISA plates. Sera were added to the sample wells, and the plates were incubated at 37 °C for 60 min. After the incubation, the mixture in the wells was discarded and the plates were washed 5 times with washing solution. Following that, chromogen A and B solutions were added to the wells, and incubation was performed again at 37 °C for 10 min in a dark environment. After the incubation, the reaction was stopped with the addition of stop solution, optical density (O.D.) values were measured using a plate reader spectrophotometer (MultiskanGO, Thermo, USA), and the results were calculated.

2.4. Calculation of fertility parameters

While fertility parameters are usually calculated as given below, pregnancy index was used as fertility parameter in the study

-Delivery to first insemination interval (days): The period from delivery to the first insemination.

-Delivery to conception interval (days): The period from delivery to conception.

-Pregnancy index: The number of inseminations made to pregnant animals / Number of animals conceived.

-First insemination success (%): Number of pregnant animals after the first insemination / number of first-time inseminated animals x 100.

2.5. Statistical analysis

Statistical comparisons were performed with SPSS software using the Mann–Whitney U, chi-square, and Fisher's exact tests at a $p < 0.05$ level of significance. For each assay, standard scores of the samples were normalized on the basis of the means as previously described [16].

3. Results

BCS values of 26 cows did not change between the pre- and postpartum periods. However, we found decreased

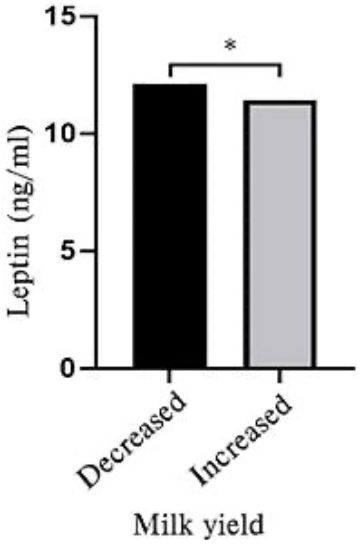

Figure 1. Prepartum levels of leptin in cows with decreased (n = 10) and increased (n = 25) milk yield in the second lactation compared to the first lactation peak (*: $p < 0.05$).

BCS in 11 of the 37 cows in postpartum period. Values of leptin, T3, T4, FT3, and FT4 were obtained in the serum samples of the study groups.

Prepartum levels of leptin decreased significantly ($p < 0.05$) in cows having increased milk production compared to the first lactation peak value. Other analyzed hormone values did not change significantly in pre or postpartum periods from the same point of view (Figure 1).

Serum FT3 levels were decreased in the postpartum period of cows having milk yield of ≥ 30 kg in the eighth week of the second lactation ($p < 0.05$). The rest of thyroid hormones and leptin did not represent a significant difference between the groups (Figure 2).

T3 concentrations were decreased in the postpartum periods of cows having non-decreased body condition score (ND - BCS). Other analyzed hormones did not alter significantly in the pre and postpartum periods in terms of BCS (Figure 3).

Serum FT3 were decreased significantly ($p < 0.05$) in the postpartum period in cows conceiving with less than 3 inseminations. However, leptin levels were increased in pre and postpartum periods of both < 3 and ≥ 3 inseminated cows to induce pregnancy (Figure 4).

4. Discussion

For a long time, studies worldwide report a reduction in fertility in high-yielding cows, most presumably based on conflicting metabolic and reproductive requirements, and one of the most prominent reproductive parameters is the number of inseminations needed per pregnancy to improve the milk production [17]. Leptin treatment has been reported to raise the secretion of LH in ruminants [18]. Likewise, enhanced endogenous secretions of the adipose tissue produced leptin, which negatively associated with extent of postpartum intervals in cattle [19]. As a consequence, it emerges as though circulating concentrations of leptin may be an indicator or a predictor of pregnancy outcome in cattle [20]. However, in this study, it was observed that serum leptin levels increased significantly in the postpartum period in Simmental cows, and this situation was more significant in cows in which the insemination number required for pregnancy was less than 3. A study conducted by Guzel and Tanriverdi [21] on some hormonal parameters in repeat breeder cows (RBC) reported lower levels of leptin in the RBC as compared to the fertile ones. Also, in another study about the plasma leptin concentration and its interrelation with the resumption of cyclic ovarian function in postpartum dairy cows, researchers reported a slightly increasing leptin levels in weeks 2 and 3 postcalving [22]. It is seen that the results reported with these studies are consistent with our findings and leptin values increase after calving and may be a predictor of pregnancy outcome in cattle.

Serum leptin concentrations are related to portion of adipose tissue, and can be associated with BCS alterations [23,24]. Some studies have reported that, the high-BCS group had the greatest plasma concentrations of leptin, whereas others have found no relationship between leptin concentrations and BCS [25]. In the present study, we could not find a relation between the serum leptin concentrations and pre and postpartum BCS. When we evaluated the study groups considering BCS due to pre and postpartum periods, it was determined that serum T3 levels significantly decreased in ND - BCS cows in the postpartum period. Hypothesizing the negative relationship between BCS and T3 and in consistence with our results, it was reported previously that, induced hyperthyroidism in Brahman cows resulted in a rise in serum T3 and was associated with a reduced BCS [26]. The alterations that we observed with thyroid hormones mostly correspond to previous studies [27,28].

Although T3, T4, and FT4 showed a tendency to reduce in the postpartum period nonsignificantly, FT3 levels decreased significantly in the postpartum period in cows conceiving with less than three inseminations. To the best of our knowledge, this study is the first to report the relation of thyroid hormones with the number of inseminations resulting in pregnancy in dairy cows. Cramer et al. [29] hypothesized that there may be an association between thyroid hormones and fertilization and mildly elevated TSH may be linked with fertilization problems in women. In a study that reported the effect of dietary energy supplementation on T3 and T4 hormones and number of inseminations in early lactation dairy cows, analysed levels

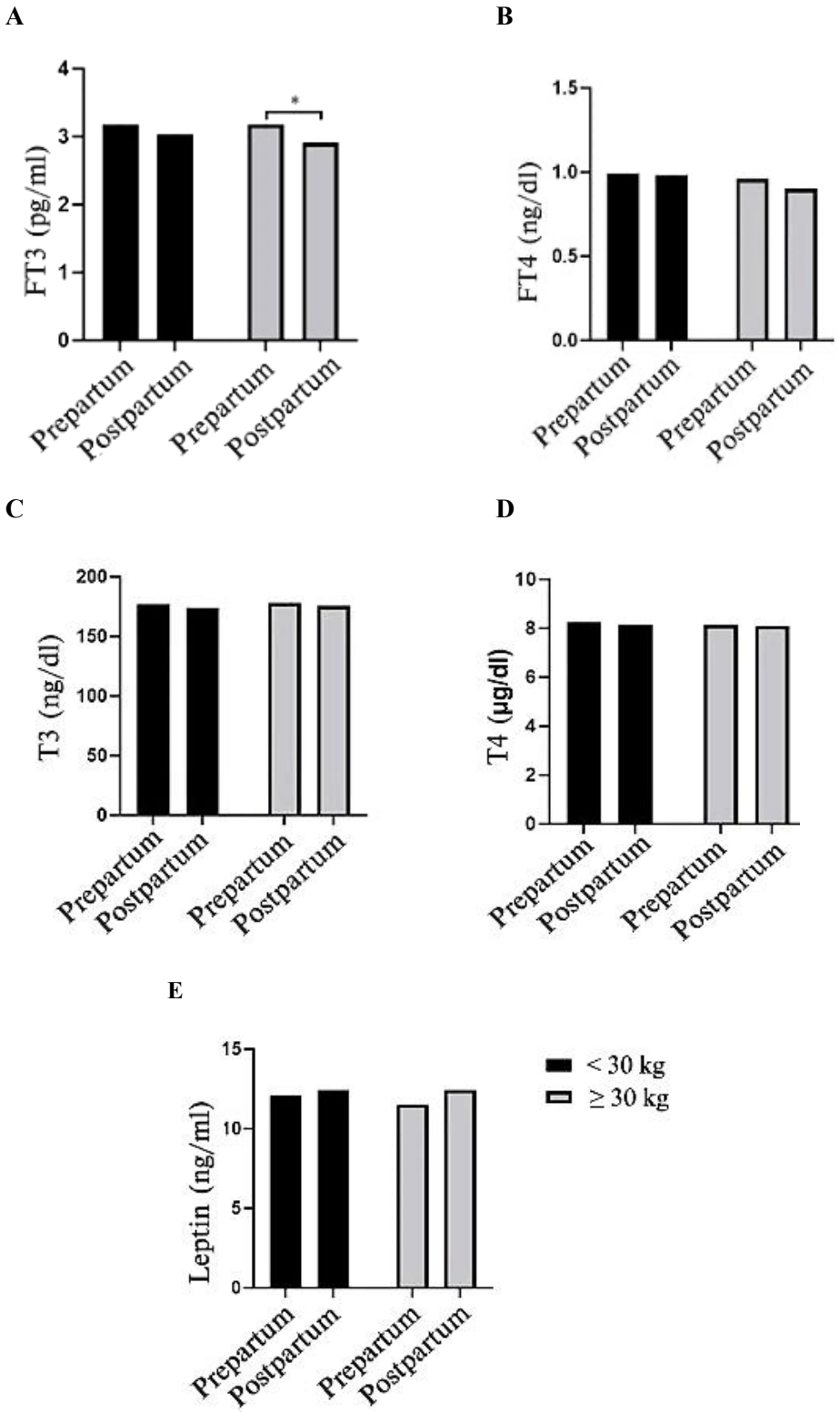

Figure 2. Serum hormone levels in the pre and postpartum periods. Cows were grouped by milk yields; < 30 kg (n = 12) and ≥ 30 kg (n = 25) in the eighth week of the second lactation (*: $p < 0.05$). A: FT3, B: FT4, C: T3, D: T4, and E: Leptin.

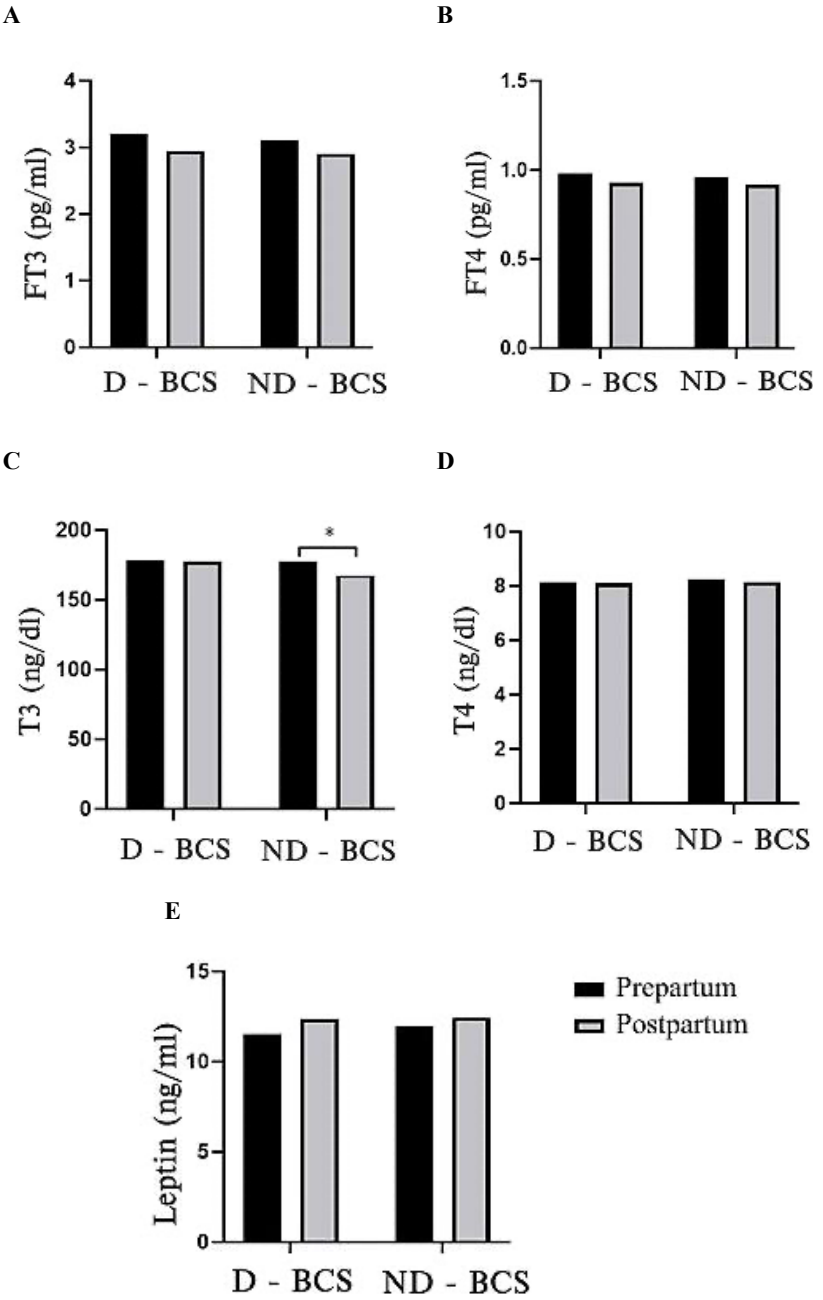

Figure 3. Serum hormone levels in the pre and postpartum periods. Cows were grouped by the decreased body condition score (D - BCS, n = 11) and non-decreased body condition score (ND - BCS, n = 26). Asterisk indicates the significant (*: $p < 0.05$) difference (Mann-Whitney U test). A: FT3, B: FT4, C: T3, D: T4 and E: Leptin.

of hormones were found to decrease in postpartum first week in comparison with the prepartum level. Authors reported average number of inseminations per conception; however, there was no data about the correlation of thyroid hormones and number of inseminations per conception [30]. Our results for T3 and T4 showed consistency with the results of Vala et al. [31] and Pethes et al. [32] who investigated the influence of nutrient management of transition period on plasma profile of thyroid hormones and postpartum fertility in buffaloes and cows. Also, Schuh et al. [27] reported that they determined lower levels of FT3 and FT4 in the postpartum third week compared to the prepartum third week in transition cows. When the results reported in literature and the findings obtained

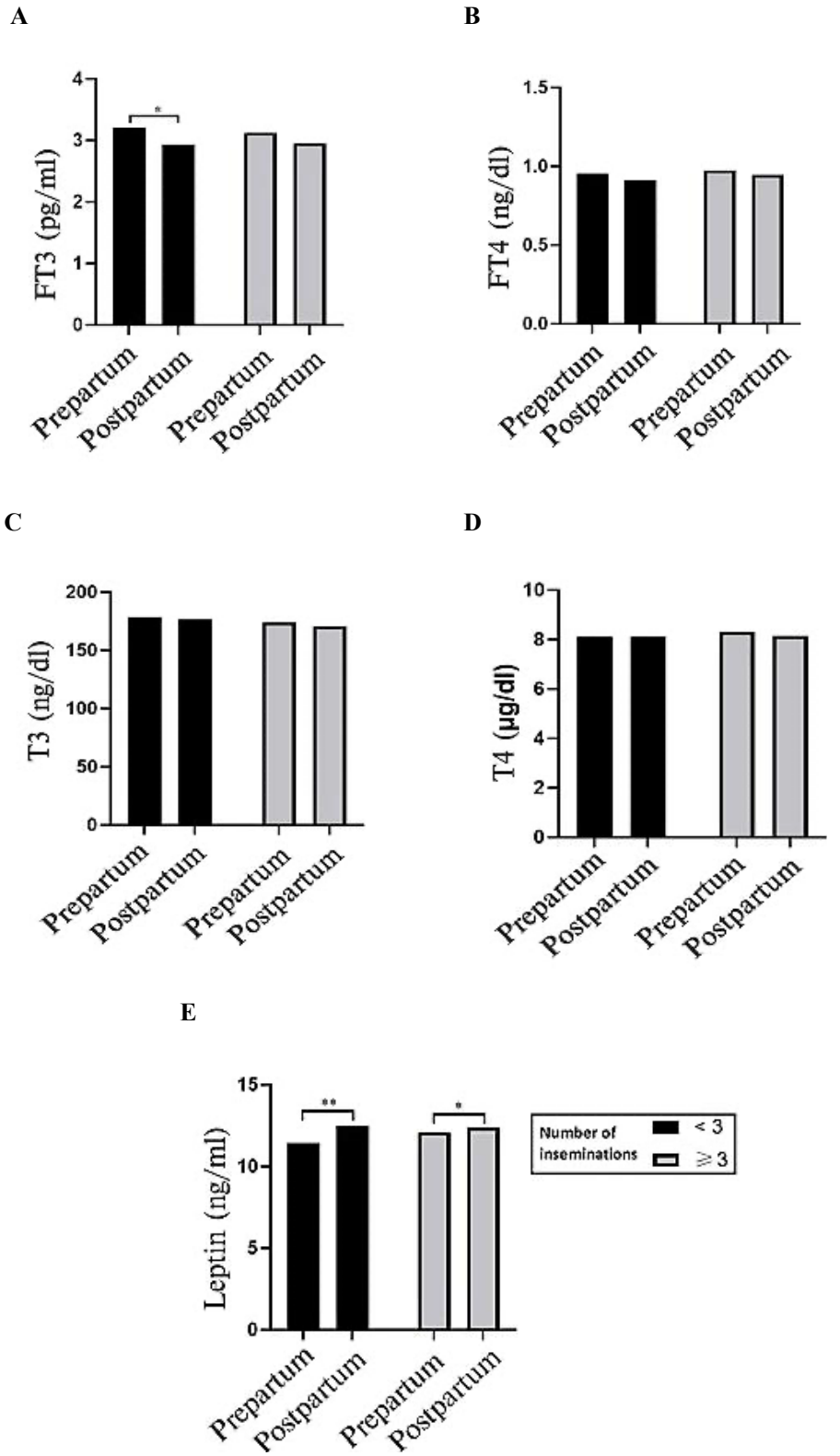

Figure 4. Serum hormone levels in the pre and postpartum periods. Cows were grouped by the total number of inseminations required per pregnancy; < 3 (n = 24) and ≥ 3 (n = 13) (*: $p < 0.05$, **: $p < 0.01$). A: FT3, B: FT4, C: T3, D: T4 and E: Leptin.

with this study are evaluated together, it can be concluded that thyroid hormones decrease slightly in 3-week period after delivery.

In the present study, we observed decreased serum FT3 levels in the postpartum period of cows having milk yield of ≥ 30 kg in the eighth week of the second lactation. As in our study, several studies [33,34] have reported a negative correlation between milk production and blood thyroid hormones in dairy cows. Thyroid hormones are necessary for the development, differentiation, and convenient metabolism of the tissues. They have a prominent effect on the readjustment of the mammary tissue and milk production. Therefore, increased levels of thyroid hormones could be determined in late pregnancy. In addition, it was reported that, reduced concentrations of these hormones could be observed during the first three months of lactation. For this reason, it may be thought that the concentration of thyroid hormones is negatively correlated with milk productivity [35]. Also, prepartum levels of leptin was decreased significantly in cows having increased milk production compared to the first lactation peak value. This result was consistent with previous studies suggesting that milk yield can affect leptin levels during lactation as higher milk production was related to lower leptin release [36,37,38].

In conclusion, as leptin influence both fat accumulation and LH levels, it may play a significant role in the courses emerging during the lactation period in dairy cows [18,19]. The presently obtained results suggest that leptin levels may be inversely related to milk production and fertility. Similarly, FT3 and FT4 levels exhibited relationship with milk production and fertility. Also, T3 hormone levels also showed an inverse correlation with BCS. In conclusion, it can be suggested that regular observation of leptin and thyroid hormone concentrations would be useful for the reproductive management of cows with breeding problems.

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Conflict of interest
No potential conflict of interest is reported by the authors.

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