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Original article

ASSOCIATION OF THYROID HORMONE PROFILE WITH RESUMPTION OF POSTPARTUM OVARIAN ACTIVITY IN DAIRY COWS

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Summary

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The objective of this study was to assess the association between thyroid hormone profile and resumption of postpartum ovulations in cattle. Lactating Holstein cows (n=40, milk yield 38.51±1.23 kg/d) were selected and based on the ultrasound results, blood estradiol and progesterone analysis, were divided into two groups. The cows were classified into anovulatory (AO) group if the first ovulation did not occur until 45 days after calving (n=16) and ovulatory group (O) if the first ovulation occurred 45 days or less after calving (n=24). Blood samples were collected from the cows weekly from day 21 to 48 postpartum to evaluate thyroid hormone levels and blood parameters. Results showed that milk yield and glucose concentrations did not differ between the groups. Serum estradiol and progesterone concentrations in ovulatory cows were significantly higher than those in AO cows. Cows in O group had better condition with respect to days to first service (DFS), open days (OD), days to first ovulation (DFO) and conception rate than cows in AO group. Thyroid hormone analysis show that T_4 concentration and T_4:T_3 ratio in the AO group were significantly higher than those in O group (P=0.001), while there were no statistically significant differences between groups for T_3 concentrations. Also, T_3 and T_4 concentrations and T_4:T_3 ratio in both groups were not affected by time (P=0.17) and time×group interaction (P=0.25). In addition, no significant difference was found in the non-esterified fatty acids (NEFA) concentration between the two groups. Unlike NEFA, beta-hydroxybutyric acid (BHBA) levels in AO group were significantly higher than those in O group (P=0.01). In conclusion, cows with different ovarian activity postpartum had different thyroid hormone profile. Increased T_4 concentration and T_4:T_3 ratio were associated with delay in ovulation and resulted in greater day to first service and open days and followed by lower conception rate

Key words: dairy cow, ovarian cyclicity, parturition, thyroid hormone

INTRODUCTION

Early resumption of the postpartum ovarian cycle in dairy cows is closely related to reproductive performance in the subsequent period. Cows that ovulated before

21 days postpartum showed better reproductive performance than cows that ovulated for the first time between days 21 and 49 (Rostami *et al.*, 2011). Most cows will have a dominant follicle within 10 to 14 days after calving; however, only 40% of these dominant follicles enter the ovulatory stage (Crowe *et al.,* 2014). In previous studies, ovulation of the first dominant follicle after calving increased estradiol pre-ovulation, whereas most cows that had no higher concentrations of estradiol did not ovulate (Beam & Butler, 1997; Butler *et al.*, 2006). A report on English cows between 1975 and 1982 showed that cows with abnormal ovarian cycles during the postpartum period had longer open days, greater insemination times, and lower conception rates at first insemination compared to normal cows (Darwash *et al.,* 1997). After calving, the intense negative energy balance and accompanying metabolic changes were associated with a decrease in fertility in high-producing dairy herds (Steinhoff *et al.,* 2019). Cows with normal ovarian cycle showed luteal activity sooner than abnormal cows and had a shorter time to first ovulation, although between these cows, a greater distance from calving to first signs of estrus was seen (Ghanem *et al.*, 2014; Sina *et al.*, 2018).

The thyroid axis in the body is one of the important axes for pregnancy in cows and metabolic adaptation during lactation. Thyroid hormones have a major effect on pregnancy and lactation (Steinhoff *et al.* 2019). Also, thyroid hormones are effective in estrus cycle, pregnancy rate, abortion and stillbirth (Silva *et al.*, 2018). The presence of triiodothyronine (T_3) and thyroxine (T_4) in the ovarian follicular fluid of farm animals and thyroid hormone receptor in human and porcine oocytes has been established (Mutinati *et al.*, 2013). Thyroglobulin (TBG) and TSH receptor have been found in bovine luteal cells (Mutinati *et al.*, 2010). On the other hand, the role of T_3 and T_4 in the steroid production in bovine follicular and luteal cells has been demonstrated (Spicer *et al.*, 2001). Thyroid hormones play an important role in fertility, tissue differentiation and foetal growth, act directly on the ovary, uterus and placenta and promote growth and metabolism through specific receptors on the growth and metabolism of these organs (Silva *et al.* 2018). Changes in thyroid hormone levels in either direction have an impact on fertility (Mintziori *et al.*, 2016).

Disruption of activity of thyroid gland causes problems in the process of germ cell proliferation and differentiation (Nikravesh & Jalaliet, 2006). This significant germ cell proliferation reduction is probably due to the disruption of gonadotropins secretion (Krassas & Pontikides, 2004). There are many conflicting data regarding the effects of hyperthyroidism on reproductive status (Krassas & Pontikides, 2004). In mice, hyperthyroidism alters placental morphology, increases its proliferative activity (Freitas *et al.*, 2007) and affects endometrial status (Kong *et al.*, 2015) Thus, deficiency in thyroid activity has many morphological and physiological effects on reproductive performance of humans and animals. The aim of this study was to investigate the relationship between thyroid hormone concentrations and ovulation in high-producing dairy cows in early lactation.

MATERIALS AND METHODS

The study was conducted at North part of Iran in Mazandaran Province, Sari (longitude °E 53.06 and latitude °N 36.33). During the study, the ambient temperature and the relative humidity of the region were 11–24 ^{o}C and 52–70%, respectively.

Animals

Multiparous Holstein dairy cows (n=40, a milk yield of 38.51±1.23, mean±SD) were enrolled into the study at day 10 postpartum. Cows averaged 3.5±1.2 (mean±SD) in parity and 3.12±0.25 (mean±SD) in body condition score at calving based on a 1 (thin) to 5 (obese) scale.

The health status of cows was examined by a veterinarian before the project began. Cows were kept alone with proper ventilation and sand bed in the postpartum period. Feed was offered as a total mixed ration (TMR) formulated according to NRC2001 recommendation 4 times daily (7, 11, 15 and 19 hours) and the same diet was fed throughout the experiment. Water was freely available.

Blood sampling and analyses

Blood samples were taken weekly from the caudal vein of all cows from day 21 to 48 postpartum (9.0 AM, one hour after morning feeding). Blood serum was then separated and stored at −20 °C until analysis.

Plasma concentrations of progesterone and estradiol β17 were measured using ELISA Reader (STAT FAX 2100). For this purpose, the ELISA Diaplace Made in Canada kit with kit numbers 4810185 and 49101123 were used. Intra- and inter-assay coefficients of variation were <5%.

Thyroid hormone concentrations in blood plasma were measured by ELISA Reader (STAT FAX 2100) using commercial kits (Orion diagnostica, Finland kit for T_3 and the Monobind ELISA kit (USA) for T_4). Intra- and inter-assay coefficients of variation were <5%. NEFA (FA 115, Randox Laboratories Ltd., Antrim, UK), and BHBA (Abbott Diabetes Care Ltd., Witney, UK) were determined by enzymatic colorimetric assays using a spectrophotometer (Shimadzu 2100, Kyoto, Japan).

Evaluation of ovarian dynamics

Ovarian follicular dynamics were monitored by ultrasound (Easy Scan, BCF Technology, and Livingston, UK) equipped with a 7.5-MHz linear rectal transducer twice weekly from day 21 to 48 postpartum to assess follicular dynamics (dominant follicle diameter, corpus luteum, and ovulation). The change in colour and shape of a large follicle from the prognosis was considered to be the dominant ovulatory follicle. One or two large follicles with a number of small follicles were considered as a follicular wave (Kafi *et al.*, 2015; Nazari *et al.*, 2019).

Cows with at least two consecutive blood samples with a progesterone concentration greater than 1 ng/mL were considered cows with luteal activity (Stevenson, 1997).

When estradiol concentrations were greater than 2 pg/mL and the dominant follicle diameter was greater than or equal to 10 mm, it was considered as the time of ovulation. If the dominant follicle diameter was greater than or equal to 15 mm, but estradiol concentrations were less than or equal to 2 pg/mL, cows were included in the anovulatory group (Cheong *et al.*, 2016).

Study groups

The base of ultrasound, estradiol and progesterone concentrations results (Kafi *et al.*, 2015), the cows were divided into the following experimental groups:

1. Ovulatory group (O): the first ovulation occurring 45 days or less after calving (n=24).

2. Anovulatory group (AO): the first

ovulation did not occur until 45 days after calving (n=16).

Statistical analysis

T_3 and T_4 concentrations in blood plasma samples were examined to investigate the relationship between thyroid hormones and different patterns of luteal activity. Data were analysed using PROC Mixed and SAS software version 9/9. Repeated Measure ANOVA was used to compare data. The statistical model of the design was as follows:

$$Y_{ijk} = \mu + T_i + A_{(i)j} + S_k + TS_{jk} + e_{ijk}$$

where: Y_{ijk} = dependent variable; μ = total mean; T_i = treatment effect; A (i) j = random animal effect in treatment; S_k = sampling time, TS_{jk} = treatment*time interaction and e_{ijk} = is the residual error (Kafi *et al.*, 2012).

RESULTS

Milk yield of groups (mean±SD) was similar (49.32±0.56 vs. 50.23±0.49 kg/d). Cows in O group had greater blood estradiol (90.92±2.46 vs. 67.56±3.53) and progesterone (24.32±1.23 vs. 12.32±2.01) concentrations compared to AO cows respectively (P<0.01). The postpartum luteal activity significantly affected DFS, OD, DFO and conception rate (Table 1) so that cows in O group had a better status compared to AO cows with respect to these parameters.

A significant difference was observed in the T_4 concentration between the two groups (P=0.006), as AO cows had higher T4 concentration (56.31±2.12 nmol/L) than ovulatory cows (42.64±3.31 nmol/L, Fig. 1). Also, in both groups, T_4 concentration was not affected by time (P=0.46) and time×treatment interaction (P=0.92, Fig. 1). The mean T_3 concentration in O and AO cows was 1.9±1.1 nmol/L and 1.96±0.02 nmol/L respectively and there was no significant difference between them (Fig. 1). Similar to T_4, time (P=0.17) and time×treatment interaction (P=0.25) did not affect T_3 concentration (Fig. 1).

The mean T_4:T_3 ratio in AO group was significantly higher (P=0.001) than in O group (Fig. 2). T_4:T_3 ratio increased significantly over time (P=0.04), while this ratio was not affected by time×treatment interaction (P=0.50).

The results of serum energy metabolites showed no statistically significant difference in NEFA concentrations between the groups (Fig. 3) while the mean BHBA concentrations in AO cows (9.34 ± 0.25 mmol/L) exceeded those in cows from the O group (7.5±0.31 mmol/L). BHBA and NEFA concentrations was not affected by time and time×treatment inter-

Table 1. Days to first service, open days, day to first ovulation and conception rate in cows with different postpartum luteal activity patterns. Data are presented as mean ± SD

Item	Postpartum luteal activity pattern	
	Ovulatory group (O) (n=24)	Anovulatory group (AO) (n=16)
Days to first service (DFS)	59.41±0.92[b]	117.32±0.75[a]
Open days (OD)	102.26±0.72[b]	167.55±0.85[a]
Day to first ovulation (DFO)	39.71±0.62[b]	95.62±0.62[a]
Conception rate (%)	69.32±0.25[a]	30.00±0.25[b]

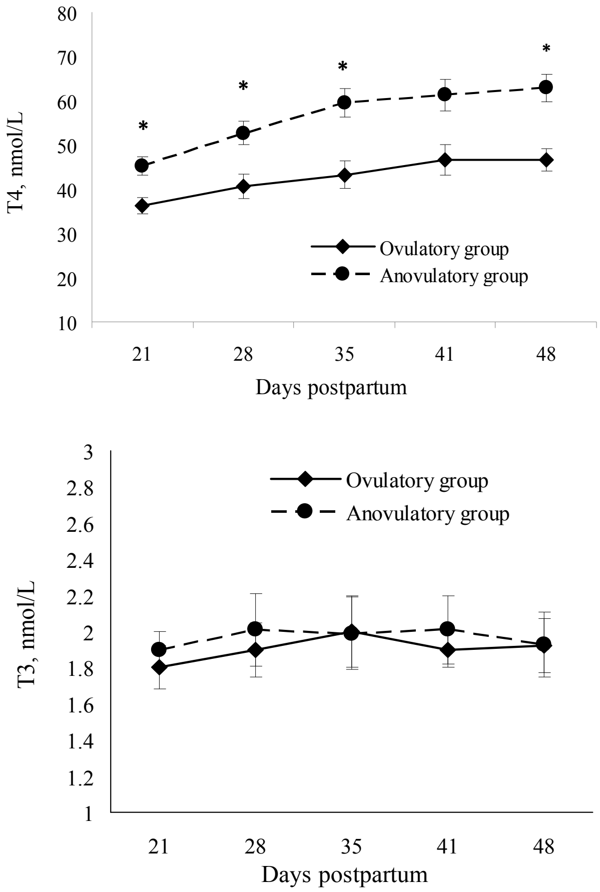

Fig. 1. Serum T_4 and T_3 levels (mean±SD) of high-producing dairy cows in ovulatory (n=24) and anovulatory groups (n=16) at different postpartum time interval (days); * P<0.05.

action in both groups. Blood glucose concentration was lower in AO compared to O group (4.16±0.37 vs. 4.5±0.32 mmol/L) but this difference was not significant (P>0.05).

DISCUSSION

The postpartum luteal activity affected significantly DFS, OD, DFO and conception rate. In line with our results, previous studies showed a significant correlation between serum progesterone profiles with a greater incidence of irregular patterns of luteal activity (Gautam *et al.*, 2010). Especially, Taylor *et al.* (2003) found a high correlation between low progesterone concentrations, long periods of anestrus postpartum and delays in uterine involution in dairy cows.

The energy and thyroid hormones levels in the PP period are the key factors that affected the early resumption of ovarian cyclicity in dairy cow after parturition (Gy *et al.*, 2002; Teixeira *et al.*, 2017; Fi-

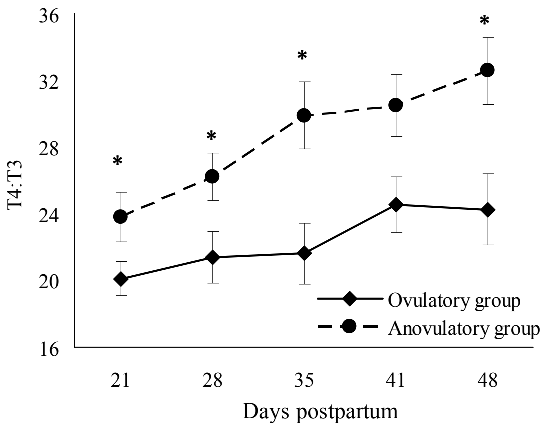

Fig. 2. Serum T_4:T_3 ratio (mean±SD) of high-producing dairy cows in ovulatory (n=24) and anovulatory groups (n=16) at different postpartum time interval (days); * P<0.05.

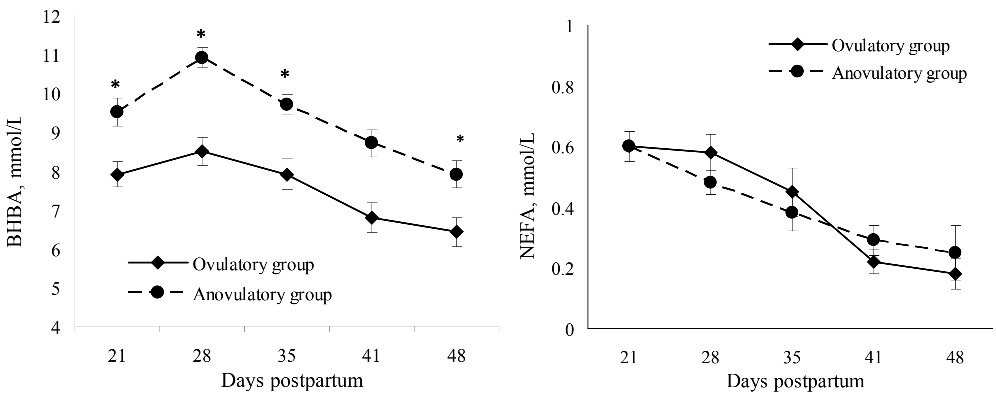

Fig. 3. Serum BHBA and NEFA concentrations (mean±SD) of high-producing dairy cows in ovulatory (n=24) and anovulatory groups (n=16) at different postpartum time interval (days); * P<0.05.

ore *et al.*, 2018). Therefore, the assessment of energy and thyroid hormones profiles can be very important for monitoring ovarian activity in postpartum cows. Based on our knowledge, a few studies have been conducted to investigate the relationship between the level of thyroid hormones and resumption of ovarian cyclicity in dairy cows after parturition (Kafi *et al.,* 2012; Teixeira *et al.,* 2017).

In this study, the cows of the ovulatory group produced the first ovulation until 40 days after parturition which was a better result than that seen in the cows of the AO group where the first ovulation did not occur until 45 days after calving. Also, the cows in the O group had significantly lower concentration of T_4 and T_4:T_3 ratio compared with the cows in the AO group. These results demonstrated a relationship between high concentration of T_4 and delayed first ovulation. Serum thyroid hormones were shown to regulate the reproduction hormones concentration (Gy *et al.*, 2002; Fiore *et al.*, 2018; Steinhoff *et al.,* 2019). An inhibitory effect of thyroid hormones on gonadotropin-releasing hormone (GnRH) and LH secretion in ewes at the transition from the breeding season to anestrus was shown (Anderson *et al.*, 2002). Hence, it could be hypothesised that the higher serum concentrations of T_4 in the postpartum period can lead to delayed first ovulation after parturition in high-producing dairy cows, due to disturbance of the GnRH and LH secretion (Kafi *et al.,* 2012). The high concentration of T_4 in AO group may be attributed to one or both of the following causes: increased production of T_4 and decreased T_4 to T_3 conversion (Kafi *et al.,* 2012). However, the second reason is more probable.

The production rate of thyroid hormones in cattle is influenced by a variety of physiological conditions. It has been proven that high producing dairy cows are in negative energy balance (NEB) during the post-partum period (Gueorguiev, 1999; Jorritsma *et al.*, 2003; Gross *et al.*, 2011). Also, the increased thyroid hormones production at that time is unfavourable due to their negative effects on physiological adjustment and milk production (Gueorguiev, 1999; Fiore *et al.*, 2017). Supposingly, in our study, the higher T_4 concentrations in the AO group compared with O group were not associated with its increased production rate. Since there was a negative correlation between thyroid hormones concentrations and milk yield and blood glucose concentration during the early lactation, uniform average milk yield and blood glucose concentrations in two study groups is one of the reasons that show thyroid hormone production rate is equal in two study groups. The significantly higher concentration of BHBA in the AO group compared with the O group confirmed this hypothesis. It is known that BHBA is one of the main serum indicators of NEB in dairy cows (Reist *et al.*, 2002; Teixeira *et al.,* 2017) with a negative correlation between BHBA and T_3 and T_4 concentrations (Jorritsma *et al.,* 2003; Kafi *et al.,* 2012; Mohebbi-Fani *et al.*, 2019). So, it was expected that T_4 concentration in the AO group was lower than that in O group while this association was not observed in our study. According to Vap & Weisser (2007) report, BHBA concentrations above 1.4 mmol/L are indicating NEB. In this study minimum concentration of BHBA concentration in AO and O groups were 7.9 mmol/L and 6.4 mmol/L respectively, proving that NEB has occurred in both groups. Several studies have reported the negative influence of BHBA on the resumption of ovarian cyclicity. Consistent with our findings, Kafi *et al.* (2012) showed that anovulatory cows had higher BHBA concentration than cows with normal luteal activity. Mohebbi-Fani *et al.* (2019) reported a positive correlation between BHBA concentration and the interval between calving to first heat. Decreased activity of the 5`-deiodinase enzyme in cows with AO was probably the major reason for the higher $T_4$ concentration in AO group compared with O group. The 5`-deiodinase enzyme converts T_4 into metabolically active T_3 in the peripheral tissues such as mammary glands and liver (Pezzi *et al.*, 2003). It has been shown the 5`-deiodinase activity can be affected by physiological conditions, for instance, Pezzi *et al.* (2003) showed a clear decrease in liver 5′-deiodinase at the peak of lactation in dairy cows. Samanc *et al.* (2010) reported that 5`-deiodinase activity was decreased in cows with severe fatty liver. Additionally, there was a negative correlation between T_3 concentration and degree of fat accumulation in liver. In our study, the comparison of BHBA between the two groups indicated that cows in the AO group presumably were more susceptible to fatty liver compared with cows in the O group. Therefore, we suggested 5'-deiodinase activity was decreased in AO group that leaded to a decreased T_4 to T_3 conversion rate of and thus, to high T_4 serum concentration and high T_4:T_3 ratio. Decreased conversion of T_4 to T_3 can affect ovary functions. In the AO group T_4:T_3 ratio showed an significant trend towards increase over time, which result probably shows that as lactation proceeds, 5`-deiodinase activity in liver was decreased. There are studies consistent with our results. Spicer *et al.* (2001) showed a highly significant association of $T_3$ levels and resumption of ovulation in dairy cows. Also, Pezzi *et al.* (2003) indicated decreased 5`-deiodinase activity at the peak of lactation in dairy cows. The study of Khal *et al.* (1987) confirmed that as lactation proceeded, there was a decrease in liver 5`-deiodinase activity.

Despite the observation that T_4 concentrations were significantly different between the two groups, there was no relevant difference in T_3 concentrations. Consistent with our finding, Kafi *et al.* (2012) showed no significant difference in T_3 concentration between cows with normal and abnormal patterns of luteal activity, whereas T_4 concentration in abnormal cows was significantly higher. Also, it is known that significant changes in peripheral tissue conversion of T_4 to T_3 may occur without a visible change in serum T_3 level (Capuco *et al.*, 1989).

CONCLUSION

In conclusion, thyroid hormone profiles in dairy cows were associated with ovarian activity postpartum. Increased T_4 concentration and T_4:T_3 ratio were associated with delay in ovulation and resulted in greater day to first service and open days and followed that lower conception rate.

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