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SERUM THYROXINE AND TRIIODOTHYRONINE CONCENTRATIONS PRIOR TO AND AFTER DELIVERY IN PRIMIPAROUS HOLSTEIN COWS

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Serum thyroxine (T_4) and triiodothyronine (T_3) concentrations were determined twice daily (8^{30}h and 16h) in seven primiparous Holstein cows from 4 days prior to (-4, -3, -2, -1) to delivery, on the day of calving, and up to 4 days postpartum (1, 2, 3, 4) by radioimmunoassay (RIA). Serum thyroid hormone concentrations were higher in the afternoon samples on the first two days of investigation (-4 and -3), but this circadian rhythm was not maintained. Serum thyroid hormones declined significantly after delivery according to a polynomial regression line (R^2=0.967 for T_4; R^2=0.9216 for T_3).

Key words: cows, delivery, thyroxine, triiodothyronine

INTRODUCTION

Thyroid gland function and hormonal changes in the different periods of the reproductive cycle in cows have been investigated by many authors (Kesler *et al.*, 1981; Aceves *et al.*, 1985; Akasha *et al.*, 1987; Nixon *et al.*, 1988; Tiirats *et al.*, 1997), and a relationship between ovarian function and the hypothalamic-pituitary-thyroid axis was proposed some time ago. It appears that plasma thyroxine (T_4) concentrations are significantly lower during the first postpartum estrous cycle than during subsequent estrous cycles (Kesler *et al.*, 1981). Our previous investigations also showed that serum levels of thyroid hormones in dairy cows decline considerably after parturition (Jovanović et al., 1988). Investigations of circadian and ultradian oscillations of plasma thyroxine and triiodothyronine (T_3) in lactating dairy cows have given evidence of rhythms in their secretion (Bitman *et al.*, 1994). The aim of the present study was: 1) to investigate the serum T_3 and T_4 concentrations in primiparous Holstein cows during the periparturient period, and 2) to reveal any possible circadian rhythm of thyroid hormone secretion.

MATERIAL AND METHODS

Serum concentrations of T_3 and T_4 were determined in seven primiparous Holstein cows before calving (1, 2, 3, and 4 days before delivery), on the day of parturition (day 0), and after calving (1,2,3 and 4 days after delivery). Two blood samples were collected at 8^{30}h and 16h. The blood serum was separated and stored at -20°C until required. Thyroid hormones in the blood serum were determined using commercial radioimmunoassay (RIA) kits (INEP-Zemun). Differences in the mean serum T_3 and T_4 concentrations prior to and after delivery, as well as between morning (8^{30}h) and afternoon (16h) samples were tested using split-plot two-factor ANOVA Factor 1= Days, Factor 2=Time and analysis of variance. Serum thyroid hormones changes were also investigated using regression analysis.

RESULTS

Serum T_3 and T_4 concentrations at 8^{30}h and 16h during the four days prior to and after delivery of primiparous Holstein cows are presented in Table 1.

Two-way analysis of variance was carried out separately for the morning and afternoon samples with day and cow as the variables. There were statistically significant differences associated with both variables ($P<0.001$), the effects being

Table 1. Morning and afternoon concentrations of the thyroid hormones in serum (nmol/L) from seven primiparous dairy cows during the puerperium.

Time postpartum (days)	T_3		T_4	
	8^{30}h	16^{00}h	8^{30}h	16^{00}h
-4	1.79^{bcd}	2.45^{a}	51.1^{c}	63.8^{a}
-3	2.09^{abc}	2.33^{a}	54.8^{bc}	64.2^{a}
-2	2.12^{ab}	2.30^{a}	63.3^{a}	60.9^{ab}
-1	2.46^{a}	2.41^{a}	59.6^{ab}	61.1^{a}
0	2.17^{a}	1.74^{cde}	59.6^{ab}	49.3^{cd}
1	1.63^{def}	1.53^{def}	42.5^{ef}	43.4^{de}
2	1.48^{def}	1.43^{def}	40.1^{ef}	41.9^{ef}
3	1.35^{f}	1.35^{f}	36.3^{fg}	33.8^{g}
4	1.38^{ef}	1.38^{ef}	33.6^{g}	38.9^{efg}
SE	0.13		2.21	
F (day)	4.99		4.15	
P-value	0.0001		0.0006	
F (time)	0.61		3.06	
P-value	NS		0.086*	
F (interaction)	2.47		4.76	
P-value	0.024		0.0002	

[abcd]Means refering to a single hormone not sharing superscripts are significantly different ($P<0.05$)

*Logarithmic transformation of the data decreased the heterogeneity of variance increasing F (time) to 4.96 ($P=0.030$)

additive for T_3 but not for T_4. Additivity indicates that the between animal differences were consistent troughout the examined time interval. Logarithmic transformation of the data for T_4 decreased their non-homogeneity of variance below the level of statistical significance without reducing the significance of the main effects which then became additive. Two missing values for each hormone on Day - 4 in the morning and one in the afternoon were calculated and the number of degrees of freedom for error adjusted accordingly.

Two-factor split-plot analysis of variance for the complete set of data (Table 1) indicated a significant influence of sampling day but not consistent diurnal effect. However, after logarithmic transformation of the data the general tendency for serum T_4 to increase during the day became statistically significant (F(time) = 4.96; P= 0.030). For individual days this difference was statistically significant on days - 4 and - 3, as well as on day - 4 for T_3. Paired t-test confirmed the significance of diurnal differences only for T_4 on day - 4 (t=2.50; P= 0.054; n=6) and the reverse on day 0 (t=3.06; P=0.022; n=7). Early morning levels of both T_3 and T_4 increased up to the day of parturition, while afternoon concentrations remained stable. These changes progressively reduced the difference in diurnal concentrations observed at the start of the experiment. Moreover, there was a significant decrease in mean serum thyroid hormone concentrations on the day of parturition. Mean levels of T_3 then tended to decline further during the first four days postpartum, but not significantly. The continued decrease in mean T_4 concentrations was more marked and statisticaly significant.

Since the changes in T_4 concentration appeared to proceed those for T_3, it may be suggested that the main influence on thyroid hormone concentrations

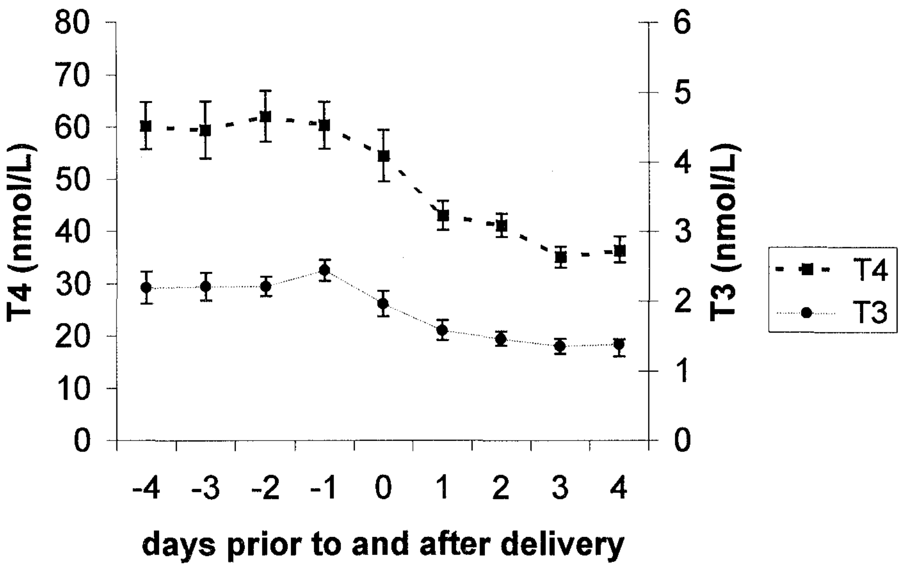

Figure 1. Serum thyroid hormone concentrations ($\bar{X}\pm$SE nmol/L) in primiparous Holstein cows before and after delivery (delivery on day 0)

was the inability of thyroid hormone secretion rate to compensate for the increased requrements associated with calving, colostrum secretion and reduced feed intake during the examined time interval.

Mean serum thyroxine concentrations declined after delivery following the polynomial regression line ($y=0.2229x^3-3.6788x^2-13.632x+48.381$; $R^2=0.967$), and the same pattern of decline is evident for the mean serum T_3 concentrations ($y=0.0102x^3-0.1667x^2+0.6412x+1.6315$; $R^2=0.9216$).

DISCUSSION

Our previous results indicated that serum thyroid hormone concentrations in dairy cows decline considerably after delivery (Jovanović *et al.*, 1988). A similar decline was reported for estradiol-17beta, but from 5-6 days to 9-10 days postpartum plasma estradiol concentrations increased. Plasma progesterone concentrations were also low during the early postpartum period, while luteinizing hormone (LH) gradually increased (Kesler *et al.*, 1979). Plasma thyroxine concentrations were suppressed during the first 2-3 weeks postpartum regardless of estrus (Kesler *et al.*, 1978). Thyroid hormone concentrations in the serum decline 2-3 times immediately after delivery, and this decline is probably not related to hypophyseal hormone secretion. In our previous work we did not detect a clear relationship between the decline of serum thyroid hormone concentrations and TSH secretion from the pituitary during the postpartal period (Jovanović *et al.*, 1988).

Frequent blood sampling in lactating cows housed in an enviromental chamber ($19\pm0.5^{o}C$, with lights on between 7-23h, fed daily at 9h, and milked at 8h and 20h) revealed circadian and ultradian changes in thyroid hormone secretion. The maximum serum concentration of thyroid hormones occured between 17-20h and the minimum between 5-13h, with peak T_4 and T_3 concentrations of 50 ± 2 ng/mL (64.35 nmol/L) and 1.58 ± 0.17 ng/mL (2.43 nmol/L), and minimal concentrations of 42.2 ± 2 ng/mL (54.31 nmol/L) and 0.94 ± 0.17 ng/mL (1.45 nmol/L), respectively (Bitman *et al.,* 1994). It is interesting to note that our data show higher serum T_3 and T_4 concentrations in the afternoon samples (16h) during the first two days of investigation (Table 1), but this difference disappeared later. Bitman *et al.* (1994) also expressed the opinion that peripheral T_3 concentration is regulated independently of peripheral T_4 concentration..

Investigating body temperature and endocrine interactions before and after delivery in beef cows, Lammoglia *et al.*, (1997) concluded that approximately 30% of the variation during the temperature decrease could be explained by plasma hormone concentrations. In their study T_3 had a negative effect on body temperature during the prepartum decrease in body temperature, and the authors offered no explanation for this phenomenon. The results of the same work showed that circulating T_3 concentrations during the precalving period were affected by the time of day: they were lowest at 3h (1.29 ± 0.03 ng/mL) and highest at 11h (1.44 ± 0.03 ng/mL) and 19h (1.39 ± 0.03 ng/mL). Plasma T_4 concentrations also tended to be affected by the time of day with the lowest values at 3h, intermediate values at 11h and the highest values at 19h (60 ± 1.4 ng/mL). Furthemore, Stewart *et al.*, (1994) found that Holstein heifers had higher plasma thyroid-stimulating hormone concentrations in the afternoon than in the morning. Our present work indicates that the patterns of change of serum thyroid hormone concentrations in primiparous Holstein cows prior to and after delivery are similar, but we could not find clear evidence for a consistant circadian rhythm of thyroid hormone secretion.

Aceves *et al.*, (1985) found that under comfortable weather conditions (temperature 22°C, relative humidity 40%) cows in early lactation had significantly lower concentrations of T_4 and T_3, and higher values of reverse T_3 (rT_3) than in dry or animals in mid lactation. In contrast, during May, when the environmental temperature increased (34°C, 40% of relative humidity) a clear-cut shift in the T_3/rT_3 ratio occurred, and animals in early lactation exhibited the highest T_3 and lowest rT_3 concentrations. In his study Tirrats (1997) showed that the plasma T_4 level was significantly lower during early lactation (45.1 nmol/L) compared with later stages, but increased as the stage of lactation progressed. Plasma T_3 concentration was significantly higher at the late stage of lactation (1.93 nmol/L) compared with the early stage of lactation (1.71 nmol/L). Levels of all thyroid hormones were negatively related to the daily milk yield. Nixon *et al.*, (1988) determined free and total thyroid hormones in the serum of Holstein cows and concluded that free and total T_4 and T_3 are low in early lactation due to the high metabolic demands for peak milk production.

Administration of bovine somatotrophin (bST) to non-pregnant lactating Holstein cows showed that the activity of thyroxine-5-monodeiodinase in mammary tissue is increased approximately twofold in response to bST administration. It was suggested that an increase in mammary conversion of T_4 to the more biologically potent thyroid hormone, T_3, plays a role in mediating the galactopoietic response of dairy cattle to bST (Capuco *et al.*, 1989). In a similar experiment in primiparous lactating Holstein cows, Kahl *et al.*, (1995) suggested that growth hormone-releasing factor and somatotrophin increase the hypothyroid status of the lactating cow but maintain a euthyroid condition in the mammary gland. Riis *et al.*, (1985) concluded that the decrease of T_4 secretion rates at the beginning of lactation seems to represent a homeorhetic adaptation to the condition of decreased energy supply. It may be a key process of adaptation of peripheral tissues to the increased metabolic demands of the mammary gland. Our results presented in this work concerning the decrease in serum thyroid hormone concentrations after delivery could be also explained by the increased metabolic demands of the mammary gland and its high priority function.

REFERENCES

1. *Aceves C, Ruiz A, Romero C, Valverde C* 1985, Homeorhesis during early lactation. Euthyroid sick-like syndrome in lactating cows, *Acta Endocrinol, (Copenh)*, 110(4), 505-09.
2. *Akasha MA, Anderson RR, Ellersieck M, Nixon DA* 1987, Concentration of thyroid hormones and prolactin in dairy cattle serum and milk at three stages of lactation, *J Dairy Sci*, 70,2, 271-76.
3. *Bitman J, Kahl S, Wood DL, Lefcourt AM* 1994, Circadian and ultradian rhythms of plasma thyroid hormone concentrations in lactating dairy cows, *Am J Physiol*, 266, 1797-1803.
4. *Capuco AV, Keys JE, Smith JJ* 1989, Somatotrophin increases thyroxine-5-monodeiodinase activity in lactating mammary tissue of the cow, *J Endocrinol*, 121,2, 205-11.
5. *Jovanović M, Stojić V, Djurdjević Dj, Sinadinović J* 1988, Puerperal changes of thyroxine and triiodothyronine serum levels in dairy cows, XIXth Annual meeting of ESNA, 29. Aug.-2.Sept,Book of abstracts, 111.
6. *Kesler DJ, Garverick HG, Bierschwal CJ, Elmore RG, Youngquist RS* 1979, Reproductive hormones associated with normal and abnormal changes in ovarian follicles in postpartum dairy cows, *J Dairy Sci*, 62, 1290.

7. *Kesler DJ, Garverick HG, Youngquist RS, Elmore RG, Bierschwal CJ* 1978, Ovarian and endocrine responses and reproductive performance following GnRH treatment in early postpartum dairy cows, *Theriogenology*, 9, 363.
8. *Kesler DJ, Johnson HD, Garverick HA* 1981, Post concentrations of thyroxine in plasma of dairy cows, *J Dairy Sci*, 64(7), 1618-20.
9. *Lammoglia MA, Bellows RA, Short RE, Bellows SE, Bighorn EG, Stevenson JS, Randel RD* 1997, Body temperature and endocrine interactions before and after calving in beef calves, *J Anim Sci*, 75, 2526-34.
10. *Nixon DA, Akasha MA, Anderson RR* 1988, Free and total thyroid hormones in serum of Holstein cows, *J Dairy Sci*, 71(5), 1152-60.
11. *Riis PM, Madsen A* 1985, Thyroxine concentrations and secretion rates in relation to pregnancy, lactation and energy balance in goats, *J Endocrinol*, 107(3), 421-7.
12. *Stewart RE, Stevenson JS and JE Minton* 1994, Serum hormones during the estrous cycle and estrous behavior in heifers after administration of propylthiouracil and thyroxine, *Domest Anim Endocrinol*, 11,1.
13. *Tiirats T* 1997, Thyroxine, triiodothyronine and reverse-triiodothyronine concentrations in blood plasma in relation to lactational stage, milk yield, energy and dietary protein intake in Estonian dairy cows, *Acta Vet Scand*, 38(4), 339-48.

KONCENTRACIJA TIROKSINA I TRIJODTIRONINA U KRVNOM SERUMU JUNICA HOLŠTAJN RASE PRE I POSLE PARTUSA

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SADRŽAJ

Ispitivanje koncentracije tiroksina (T_4) i trijodtironina (T_3) u krvnom serumu vršena su radioimunološkom (RIA) metodom kod 7 junica Holštajn rase 4 dana pre porođaja (-4, -3, -2, -1), na dan porođaja (0 dan), i posle porođaja (1, 2, 3, 4 dana) uzimanjem dva uzorka krvnog seruma dnevno (u 8^{30}h ujutro i 16h popodne). Koncentracija hormona tireoideje bila je viša u popodnevnom uzorku u prva dva dana ispitivanja (- 4 i - 3 dana), ali nije ustanovljen jasan cirkadijalni ritam njihove sekrecije. Koncentracija hormona tireoideje opada značajno nakon partusa prateći regresionu polinomsku liniju (R^2=0.967 za T_4; R^2=0.9216 za T_3).