Global Veterinaria 12 (1): 59-66, 2014
ISSN 1992-6197

DOI: 10.5829/idosi.gv.2014.12.01.81242

Leptin, Thyroxin and Cortisol Hormones and Some Metabolic Products During Pre and Postpartum Periods in Cows in Relations to Their Body Weight of Newborn Calves

F.E.I. Teama and A.E. Gad

Department of Biological Applications, Radioisotopes Applications Division,
Nuclear Research Center, Atomic Energy Authority, Inshas, Cairo, Egypt

Abstract: Relationships among plasma hormonal and metabolic variables in pre and postpartum of gestation in 10 crossbred dams and the BW of their offspring were investigated. Ten pregnant crossbred (Brown Swiss x ballady) cows were monitored 4 weeks pre and postpartum periods to study the levels of leptin, thyroxin (T4) and cortisol hormones levels as well as some metabolites during pre and postpartum periods and their relationships with calf birth weight. Blood samples were collected at -4,-3,-2,-1 week, before and +1,+2,+3,+4 weeks after parturition to assess leptin, thyroxin (T4) and Cortisol hormones levels in addition to glucose, NEFA, B- HBA, A significant decrease of glucose level and Leptin, T4 and Cortisol hormones were recorded. There was highly significant increase in NEFA, B- HBA, concentrations as effected by postpartum period. There is a significant positive correlation between calf birth weight and NEFA, B- HBA, this means that the energy metabolites is a good indicator to calf body weight and health.

Key words: Hormones · Energy metabolites · Newborn birth weight · Crossbred cows

INTRODUCTION

Pregnancy and lactation are physiological status considered to modify metabolism in animals and induce stress [1, 2]. The preparturent period is important in terms of its influence on the animals and the subsequent performance of cows, since cows develop serious metabolic and physiological changes during these periods [3]. In fact, it is well known that during the pregnancy all the metabolic pathways are involved in sustaining the fetus growth [4]. The period of transition between late pregnancy and postpartum presents a huge metabolic challenge to the cow and the haematochemical profiles are important in evaluating the animal's status during this period [5]. Immediately after the calving, there is a status of severe negative energy balance, indicated by alterations in blood metabolite and hormone profiles [6]. Specifically, high levels of non-esterified fatty acids (NEFA) and β-hydroxybutyrate concentrations are indicative of lipid mobilization and fatty acid oxidation [6] Also, there are numbers of hormones thatpotentially direct or indirect regulators of feed intake including reproductive hormones, stress hormones and leptin [7].

The main objective of the present study was to investigate the level of different metabolites such as glucose, NEFA and β-HBA and leptin, T4 and cortisol hormones during pre and postpartum period of cows in relations to body weight of newborn calves.

MATERIALS AND METHODS

Design of Experiment, Animal Feeding and Management: The experiment was performed using ten multiparous crossbred pregnant cows (Brown Swiss x Ballady) considering parity (3: 4) chosen according to the nearest calving day and from the bovine farm of "Experimental farms project" of Nuclear Research Center, Atomic Energy Authority of Egypt which is located in the desert area of Inshas. The average body weight was 391 ± 2 Kg. The cows were monitored from 4 weeks before and after parturition and the baby calves were weighted at delivery time. The cows were housed in a free-stall barn with rain force shade during day and night. The cows were offered the same concentrate basal diet before and after parturition contained 2 %/h /daily according to the average body weight. The concentrate consisted of 27%

Corresponding Author: Fatma EI Teama, Department of Biological Applications, Radioisotopes Applications Division, Nuclear Research Center, Atomic Energy Authority, Inshas, Cairo, Egypt.

undecorticated cotton seed meal, 35% wheat bran, 30% yellow corn, 5% soybean meal, 1.5% lime stone and 1.5% sodium chloride. Rice straw available all time. Minerals and vitamin block (Tithebarn limited, Wins ford, Cheshire CW7 3PG.U.K.) were available for all cows during all times. The animals were watered freely *ad libitum*.

Blood Samples and Assays: The blood samples were taken every one week intervals -4, -3, - 2, - 1 before expected calving and +1,+2+,+3 and +4 weeks post calving. The blood from each cow was collected from a jugular vein directly into three separate test tubes, without anticoagulant for serum to determined Hydroxybutyrate (β -HBA), Non-Esterified fatty acids (NEFA) and hormones. Sodium fluoride tube for glucose and heparenized tube for plasma leptin. The tubes were placed in a mobile refrigerator at 4°C to transfer to the laboratory and then centrifuged for 30 min at 3000 rpm at 4°C. Serum was separated in vials and kept at-20°C until analyzed. Glucose was determined using commercial kit (Biodiagnostic, Egypt). Β-Hydroxybutyrate (BHBA) and Non-esterified fatty acids (NEFA) were determined by a D-3-hydroxybutyrate kit and a NEFA Kit (Randox Laboratories Ltd, Ardmore, UK). Thyroxin (T_4) and cortisol levels were estimated by the radioimmunoassay (RIA) technique using solid phase coated tubes kits (I^{125}) (Diagnostic Systems Laboratories, Inc. Webster, Texas, USA). The quantitative measurement of leptin hormone in plasma was performed using ELISA kit for multispecies (DRG Diagnostics, Marburg, Germany) according to the manufacturer's instruction.

Statistical Analysis: Data were statistically analyzed using the general linear model procedure of GLM anova procedure by SPSS (V. 8). The model used is: $Yij = (\mu + P_i + Tj + (PT)_{ij} + eijk$ where (μ = the overall mean; Pi = the fixed effect of the physiological period (Prepartum and postpartum), Tj = the fixed effect of weeks; (PT)ij=the interactions between the two factors (P*T) and eijk = random error. Significance of the difference between the means was verified by Duncan's [8].

RESULTS

Hormonal levels: Leptin concentrations in crossbred cows tended to present steeper prepartum decay and reached a lower level during postpartum. There was a highly significant ($P < 0.0001$) effect between the two physiological periods (Pre and postpartum), with leptin levels become down in pre calving (-1 week than -4week) and continued after calving. There was a significant effect of weeks ($P < 0.005$) and interaction between physiological periods versus weeks ($P < 0.0001$). Table (1) includes (Mean ±SE) of weekly leptin concentrations by 4 weeks before and after calving. The concentration of leptin was reduced by 20.9% during

Table 1: Leptin, T4 and Cortisol hormones in crossbred cows in pre and postpartum (Mean ±SE).

	Hormones		
Items	Leptin(ng/ml)	T4 (nmol)	Cortisol(nmol)
Physiological period (Phys.)			
Prepartum	3.83±0.12^A	66.1±0.50^A	16.05±0.32^A
Postpartum	3.03±0.08^B	56.2 ±0.40^B	2.39 ±0.10^B
Significance (p=)	0.0001	0.0001	0.0001
Time of sampling (week, T)			
1st	3.11±0.2^B	59.21±2.5^c	11.03 ±0.25^A
2nd	3.34±0.3^{AB}	60.33±2.8^{BC}	9.44 ±0.28^B
3rd	3.57±0.6^A	61.49±2.6^B	8.41 ±0.28^C
4th	3.62±0.5^A	64.20 ±3.1^A	8.01 ±0.26^C
Significance (p<)	0.005	0.0001	0.0001
Interactions(Phys. *T)			
prepartum			
1st	3.30±0.10^a	61.70±0.10^d	19.28±0.31^a
2nd	3.64±0.10^b	68.00±0.07^d	16.31±0.51^{ab}
3rd	4.13±0.20^a	67.10±0.10^d	15.10±0.47^{bc}
4th	4.30±0.17^a	68.00±0.08^d	14.51±0.44^c
Postpartum			
1st	2.94±0.12^c	56.71±0.41^c	2.78±0.10^d
2nd	3.03±0.13^c	52.65±0.65^c	2.56±0.07^d
3rd	3.01±0.11^c	55.87±0.60^c	1.71±0.10^d
4th	3.15±0.08^c	60.03±0.38^b	2.51±0.08^d
Significance (p<)	0.0001	0.0001	0.0001

Means bearing different letters (A, B and Cor a,b,c and d) in the same column within each classification differ significant (P<0.05or0.01),respectively.

Table 2: Glucose, NEFA and B-HBA in crossbred cows in pre and postpartum period (Mean±SE).

	Biochemical parameters		
Items	Glucose(mg/dl)	NEFA (umol/L)	B-HBA (umol/L)
Physiological period			
Prepartum	65.33 ± 1.8 A	320.04±6.2 B	422.80±6.2 B
Postpartum	54.41 ±1.6 B	526.65 ±11.3 A	576.57±9.1 A
Significance (*p*<)	0.0001	0.0001	0.0001
Time of sampling (week)			
1st week	55.84±5.2C	547.24 ±7.9A	651.8±7.9A
2nd week	58.4±5.8BC	480.82 ±8.6A	531.6 ±6.5B
3rd week	60.87±5.9B	365.34 ±8.2B	456.7±7.10C
4th week	63.87±5.5A	299.98 ±8.3B	358.8 ±9.7D
Significance (*p*<)	0.0001	0.0001	0.0001
Interactions(p*T)			
prepartum			
1st	61.01±1.25 c	393.65±6.59 a	569.5±7.10 b
2nd	64.19±1.39b	306.73±6.61d	424.1±4.01c
3rd	66.70±1.40a	290.39±5.87d	429.6±4.93c
4th	69.42±1.39a	289.38±5.96d	268.2±8.14c
Postpartum			
1st	50.66±0.61f	700.83±10.6a	734.1±8.72a
2nd	52.64±0.45f	654.91±10.4b	639.0±9.10ab
3rd	54.90±0.42d	440.28±11.3c	483.8±5.6c
4th	58.32±0.92c	310.59±11.8d	449.4±5.02c
Significance (*p*=)	N.S.	0.0001	0.0001

Means bearing different letters (A, B andC or a,b,c,d and f) in the same column within each classification differ significant ($P< 0.01$), respectively. N.S. =not significant.

Table 3: Correlations between body weight of newborn calves and metabolites in prepartum period

Variables						
Birth weight	Glucose	NEFA	B-HBA	T4	Leptin	cortisol
1st	0.025	0.529*	0.407	0.282	-0.345	-0.280
2nd	0.395	0.440	0.220	0.103	0.098	-0.208
3rd	0.395	0.431	0.301	-0.187	0.273	-0.455
4th	0.394	0.336	0.682*	0.070	0.065	-0.499

*. Correlation is significant at the 0.05 level.

postpartum period. Inspection of the leptin profile suggests that this reduction was initiated a few days before parturition. The depression in the concentration of plasma leptin persisted at week +4.

For the current study, serum T4, prepartum was decreased before calving by one week pre and 3 weeks postpartum, the differences between two periods were 15% and significant higher ($P< 0.0001$). This decrease following parturition and continued to week 4. Physiological period, time of sampling effect (T) and week versus period (P*T) interaction were significant higher ($P < 0.0001$) Table 1.

Table 1 indicated that the prepartum period had a greater mean of cortisol, a decrease in serum cortisol sustain from time immediately following parturition until the end of experiment. This difference between two physiological periods was 85.1% and the interaction of period versus week was significant higher ($P < 0.0001$) with significant main effect of week.

Metabolite Levels: Glucose mean level between both period had a highly significant effect ($P <0.0001$). At first week postpartum, the average had dropped by 17.2%. Although there was significant effect of weeks and no significant effects for period versus week's interaction (P*T), as showed in Table 2.

There was a significant effect of period (P) and weeks (T) in NEFA level and interaction between physiological periods versus weeks was significantly differences by ($P <0.0001$) as showed in table 2. After parturition, first week NEFA levels were on average greater than the prepartum. However, week 1 postpartum the NEFA mean levels rose by about 78% than that in prepartum period. Postpartum period had a general trend of gradual decline in NEFA concentration from week 3 to week 4 postpartum.

Table 2 showed an increase in mean serum of β-HBA levels within the first week before and after parturition and this increase was significant at ($P <0.0001$). β-HBA at parturition, rose sharply to approximately 2 weeks postpartum and slowly decreased thereafter (Table 2).

Our cows had β-HBA levels with significant different (P<0.0001) between the two period, weeks and period x weeks interaction.

Correlation Coefficient Between Metabolites of Cows and Body Weight of Their Newborn: All of the cows delivered a singleton calf (6males and 4 females), the mean of birth weight (BW) of newborn was 25.80±0.70 kg; the heaviest calf was male for dam cows with greater NEFA level. There are a positive correlation between the newborn BW and NEFA, β-HBA and T4. On the other hand, Negative correlation found between the newborn BW and leptin at the first week prepartum and cortisol hormone at last 4weeks prepartum. NEFA were found the ones most closely correlated with calf birth weight Table 3.

DISCUSSION

Hormonal Levels: The pregnancy and lactation phases affect significantly the metabolic profile and so the variation recorded during different physiological phases is discussed because the transition from gestation to lactation is a period of great metabolic stress for cows [9]. In our study, Leptin is a protein hormone produced and secreted primarily by white adipocytes and has been shown to be a potential regulator of feed intake. It may play a role as a signal to central nerves system (CNS) indicating energy status of the animal [10]. Because of its role in the regulation of feed intake and energy deposition, it could also participate in the co-ordination of metabolism during the transition from pregnancy to lactation. It was reported that reduction in the plasma concentration of leptin is associated with the transition from pregnancy to lactation [11]. During postpartum and early lactation, cows that are in a more sever negative energy balance have a greater mobilization rate of NEFAs from adipose tissue as a result, adipocytes are depleted and leptin synthesis is reduced [10]. On the other hand, previous study done by Soliman and colleagues [12] showed a relatively constant serum leptin level during the preparturent period. This is in conflict with our present study and others. Our study is in line with the observation of Blok and colleagues [10], [13 and 14]. Increased level of circulating leptin form early to mid-pregnancy until late Pregnancy was recorded in sheep [15, 16] which attributed to an increasing in adiposity as an increasing in leptin mRNA expression in adipose tissues [16]. In ruminants, studies have yet to be performed to investigate the changes in the long form of leptin receptor in hypothalamus during pregnancy. Plasma leptin concentration increases significantly during pregnancy which due to the production of leptin by the placenta [16, 17] and related with the well being of the fetus.

The function of the mammalian thyroid gland during the transion period received much attention during the past few years. However, during gestation, thyroid hormones may play a role in maintenance of pregnancy and during lactogenesis. It may be an important hormone for normal ductal development of the mammary gland. Additionally, higher levels of thyroid hormones may act as a metabolic signal to the cow to begin resumption of ovarian activity [18]. The present study shows significant difference between the two periods (Pre and postpartum). T4 hormone was affected by weeks postpartum as shown in results. Concentrations started to decrease before parturition and minimum levels were found soon after parturition. Thyroid T4 hormone did not recover postpartum concentrations until the end of the week 4. Decrease in thyroid hormones around parturition due to decrease feed intake and alterations in cardiac output and increased blood volume [19, 20].

The decrease in T4 may support the mammary gland in partitioning of nutrients between mammary and non-mammary tissue. This decrease in secretion of T4 decreases the adverse effect of nutrients deficiency in body tissue at the onset of lactation.This more exaggerated decline in maternal T4 levels could influence mammary tissue development, postpartum milk yield and fetal growth [21].

Cortisol is the major glucocorticoid produced by the adrenal cortex in response to stressful stimuli and also plays a role in glucose regulation. Several previous studies have shown that at the time of parturition, cortisol peaks, followed by a sharp drop in concentrations in early lactation [22]. Over time, cortisol synthesis will be increased. Our results agree with previous studies, where cortisol hormone level was drop to nadir levels in postpartum period by 85.1% until the end of experiment. For many years glucocorticoid have been used in the medicine for their immunosuppressive effects on the immune cells that are suppressed in postpartum cows and because cortisol peaks are found at the time of parturition, the cow's immune system is compromised [22, 23].

Metabolite Levels: The metabolic state of the cow may also be described through the levels of plasma metabolites related to the energy metabolism.

Glucose is important to the high demands of energy for the processes of lactation and maintenance, glucose has been shown to be depressed in several studies in the early postpartum period, thereafter levels beginning to increase. This coincides with the cow's decrease in feed intake at parturition and a progressive recovery in appetite and energy status [24]. Our results are consistent with this earlier study reported. In the present study, at postpartum there was an acute decrease in plasma glucose for gluconeogenesis, presumably due to the energy demands of the mammary gland. The large demand for glucose may decrease the amount of glucose available to other tissues in the body, including those that that are involved in postpartum reproduction [25, 26]. Previous study disagrees with the present data [27], where they found that the plasma glucose concentrations were greater during the early postpartum period.

It was previously reported that elevated NEFA concentrations around the time of calving and early lactation are inversely related to glucose and insulin concentrations [28]. The increase in plasma NEFA level during late gestation might be due to an increased nutrient requirement caused by the growth of a fetus. Therefore, body fat reserves may be mobilized and, as a consequence, plasma NEFA content increased close to the end of gestation [29]. This situation may be further proed by a large size of the fetus relative to its dam; this could lead to a decrease in dry matter intake by the dam because of reduced space availability in the digestive tract caused by the size of the calf [30].

For the Postpartum period, elevated serum NEFA concentrations would seemingly agree with a more elevated plasma β-HBA concentration. Elevated NEFA may also have an effect on the oviduct and uterus. Wathes and colleagues [25] have recently shown that cows in severe negative energy balance experience changes in the IGF and insulin signaling pathways that could affect the uterus that is undergoing extensive repair after calving. These changes in growth factor signaling pathways may also limit fetal-uterine interaction necessary for optimum fetal growth and development. This finding disagrees with the results obtained by Garverick and colleagues [27].

Increased plasma NEFA concentrations, as showed during the postpartum, are useful for the animals to maximize milk synthesis with lower glucose consumption, moreover, the high Growth hormone concentrations and the low insulin levels, present in blood stream during this period, stimulate a marked mobilization from adipose tissues, as confirmed by the increase in NEFA plasma levels [31, 32]. Ketosis commonly results either from the lack of sufficient glucose precursors available for energy production or from a reduced gluconeogenic capacity by the liver and it is characterized by elevated concentrations of the ketone bodies acetoacetate, acetone and β-hydroxybutyrate in the blood, milk and urine [33].

Bertics and colleagues [34] reported that as a result of decreased feed intake around the time of parturition, an important metabolic response in the cow is to mobilize triglycerides (TG's) from adipose tissue to the liver to provide enough energy for the rapid fetal growth in late gestation, lactogenesis and maintenance functions. Many studies have shown that due to the increased mobilization of adipose tissues, the NEFA levels are increased around the time of parturition, which agrees with the present results [34, 25].

During the drop in feed intake around the time of parturition, glucose reserves are depleted thus resulting in increased ketone levels. With increased energy demands for both maintenance functions and lactogenesis, the ketone β-HBA tends to increase following day of calving until about 3 weeks postpartum. Thereafter, levels begin to decline as the cow recovers from hypophagia and a negative energy balance [24]. In our results the mean weekly serum β-HBA is in agreement with the results obtained by Vasquez-Anon and colleagues [24]. The ketone level gradually increased following day of calving up until week 2. The significant differences between the two periods in their levels of plasma β-HBA indicate that the cows may have experienced a greater drop in preparturent feed intake. However, this conclusion cannot be confirmed since feed intake was not measured in the current study and the cows were received the same rotation without any additives to maintain the physiological status. An increased concentration of β-HBA is a sign of negative energy balance [37]. Therefore, the cows may have been in a greater negative energy balance in early lactation. Previous studies have not shed light on whether or not ketone body production is altered under this condition. It seems that the level of β-HBA is directly related to feed intake and is inversely related to glucose availability [7].

NEFA and BHB levels increased around parturition, reflecting the negative energy balance (NEB) of the animals and indication that mobilization of body tissue comprised not only fat but also muscle tissue and agree with Ingvartsen and Andersen [7] and Kessel and colleagues [35]. Cows with greater NEFA are presumably mobilizing more adipose tissue to support milk production and are losing more body weight compared with cows with lesser NEFA [36]. The cows had lower body weight was consistent with the higher NEFA levels and the greater number of BHB. This is probably related to the increased the simultaneously with the demands of lactation and their lower feed intake capacity as described previously [37].

Correlations Between Newborn Body Weight (Bw) and Some Metabolites of Their Dams During Prepartum Period: In the present study a positive correlation was recorded in the between the newborn BW with each of NEFA at 1st week but not power, β-HBA significant at week 4. On the other hand, our results are agree with Echternkamp [38] and Zhang and colleagues [39] have reported a positive relationship between maternal some metabolites, some plasma hormones and calf BW. Lipomobilization increased with calf birth weight during the last 4 weeks of pregnancy [40]. This means that NEFA is a good indicator to calf body weight and health and could be interesting for detecting eventual problems related to an excess of mobilization of body lipid reserves of the dam. The reason for this relationship is unclear and its confirmation and interpretation requires further investigation.

CONCLUSION

It could be concluded that there are a huge changes in some metabolites during pre and postpartum in crossbred cows (Brown Swiss X Ballade) such as NEFA and β-HBA and are assumed to be the best indicators of a cows energy balance [41]. The NEFA were found the ones most closely correlated with calf birth weight. The forthcoming birth of a calf with a heavy BW seems to be preceded by a pronounced increase in NEFA and β-HBA levels in the dam just around the time of parturition. We recommended need more study about lipids and lipids oxidation in prepartum period to detect which types are good markers to newborn body weight and mother cow health and performance.

REFERENCES

1. Iriadam, M., 2007. Variation in certain haematological and biochemical parameters during the peri-partum period in Kilis does. Small Ruminant Research, T., 73: 54-57.
2. Tanritanir, P., S. Dede and E. Ceylan, 2009. Changes in some macro minerals and biochemical parameters in female healthy siirt hair goats before and after parturition. J.Anim. and Vet. Advanc., T., 8: 530-533.
3. Tanaka, M., Y. Kamiya, T. Suzuki and Y. Nakai, 2011. Changes in oxidative status in periparturient dairy cows in hot conditions. J. Anim. Sci., T., 82: 320-324.
4. Bell, A., W.S. Burhans and T.R. Overton, 2000. Protein nutrition in late pregnancy, maternal protein reserves and lactation performance in dairy cows. Proceedings of the Nutrition Society, T, 59: 119-126.
5. Hawagane, S.D., S.B. Shinde and D.N. Rajguru, 2009. Haematological and blood biochemical profile in lactating buffaloes in and around Parbhani city. Veterinary World, T., 2: 467-469.
6. Wathes, D.C., Z. Cheng, W., Chowdhury, M.A. Fenwick, R. Fitzpatrick, D.G. Morris, J. Patton and J.J. Murphy, 2009. Negative energy balance alters global gene expression and immune responses in the uterus of postpartum dairy cows. Physiologcal Genomics, T, 39: 1-13.
7. Ingvartsen, K.L. and J.B. Andersen, 2000. Integration of metabolism and intake regulation: a review focusing on perparturient animals. J. Dairy Sci., 83: 1573-1597.
8. Duncan, D.B., 1955. Multiple range and multiple F- test. Biometrics, 11: 1-42.
9. Rollin E., R.D. Berghaus, P. Rapnicki, S.M. Godden and M.W. Overton, 2010. The effect of injectable butaphosphan and cyanocobalamin on postpartum serum B-Hydroxybutyrate, calcium and phosphorus concentrations in dairy cattle. J. Dairy Sci., T., 93: 978-987.
10. Block, S., W. Butler, R. Ehrhardt, A. Bell, M. Van Amburgh and Y. Boisclair, 2001. Decreased Concentration of Plasma Leptin in Periparturient Dairy Cows is caused by Negative Energy Balance. J. Endocrin, 171: 339-348.
11. Woodside, B., A. Abizaid and C. Walker, 2000. Changes in leptin levels during lactation: implications for lactational hyperphagia and anovulation. Hormones and Behavior, 37: 353-365.
12. Soliman, M., K. Ishioka, R. Yoshida, K. Komabayashi, H. Hatai, Y. Matsui, T. Hirai, S. Katagiri, Y. Takahashi, Y. Kawakita, H. Abe, H. Kitamura, K. Kimura and M. Saito, 2002. Serum Leptin Levels during the Periparturient Period in Cows. J. Vet. Med. Sci., 64(11): 1053-1056.
13. Chilliard, Y., C. Delavaud and M. Bonnet, 2005. Leptin expression in ruminants: Nutritional and physiological regulations in relation with energy metabolism. Dom.Anim.Endocrinol, 29: 3-22.

14. Hachenberg, S., C. Weinkauf, S. Hiss and H. Sauerwein, 2007. Evaluation of classification modes potentially suitable to identify metabolic stress in healthy dairy cows during the peripartal period.J.Anim. Sci., 85: 1923-1932.
15. Forhead, A.H., L. Tomas, J. Crabtree, N. Hoggard and D.S. Gardner, 2002. Plasma leptin concentration in fetal sheep during late gestation: Ontogeny and effect of glucocorticoids, Endocrinology, 143: 166-1173.
16. Ehrhardt, R.A., R.M. Slepetis, A.W. Bell and Y.R. Boisclair, 2001. Maternal leptin is elevated during pregnancy in sheep, Domest Anim. Endocrinol, 21: 85-96.
17. Masuzaki, H., Y. Ogawa and N. Sagawa, 1997. Nonadipose tissue production of leptin: leptin as a novel placenta-derived hormone in humans. Nat. Med., 3: 1029-1033.
18. Reist, M., D. Erdin, D. von Euw, K., Tschuemperlin, H. Leuenberger, C. Delavaud, Y. Chilliard, H.M. Hammon, N. Kuenzi and J.W. Blum, 2003. Concentrate feeding strategy in lactating dairy cows: metabolic and endocrine changes with emphasis on leptin. J.Dairy Sci., 86: 1690-1706.
19. llek, J., N.F. Khaled, D. Sedlakova and M. Sindelar, 1998. Concentration of thyroid hormones in dairy goats during lactation. In: 6th International symposium on the milking of Small Ruminants, Sept 26- Oct 1, Athens, Greece.
20. Khaled, N.F., 2001. Interactions between nutrition, blood metabolic profile and milk composition in dairy goat. Ph.D thesis, VFU, Brno, Czech Repu, pp: 227.
21. Collier, R., D. Beede, Thatcher, L. Israel and C. Wilcox, 1982. Influences of Environment and its Modification on Dairy Animal Health and Production. J. Dairy Sci., 65(11): 2213-2227.
22. Goff, J.P. and R.L. Horst, 1997. Physiological changes at parturition and their relationship to metabolic disorders. J. Dairy Sci., 80: 1260-1268.
23. Le Blanc, S., 2010. Monitoring metabolic health of dairy cattle in the transition period. J. Reprod. Dev., 56(Suppl.): S29-S35.
24. Vazquez-Anon, M., S. Bertics, M. Luck, R. Grummer and J. Pinheiro, 1994. Peripartum Liver Triglyceride and Plasma Metabolites in Dairy Cows. J. Dairy Sci., 77: 1521-1528.
25. Wathes, D.C., Z. Cheng, M.A. Fenwick, R. Fitzpatrick and J. Patton., 2011. Influence of energy balance on the somatotrophic axis and metalloproteinase expression in the endometrium of the postpartum dairy cow. Reproduction, 141: 269-281.
26. Green, J.C., J.P. Meyer, A.M. Williams, E.M. Newsom, D.H. Keisler and M.C. Lucy., 2012. Pregnancy development from day 28 to 42 of gestation in postpartum Holstein cows that were either milked (Lactating) or not milked (Not lactating) after calving. Reproduction, 143: 699-711.
27. Garverick, H.A., M.N. Harris, R. Vogel-Bluel, J.D. Sampson, J. Bader, W.R. Lamberson, J.N. Spain, M.C. Lucy and R.S. Youngquist, 2013. Concentrations of nonesterified fatty acids and glucose in blood of periparturient dairy cows are indicative of pregnancy success at first insemination. J. Dairy Sci., 96: 181-188.
28. Nordbladh, L.I., A.E. Sweetman and C.S. Whisnant, 2003. Postpartum changes in hormones and metabolites during early lactation in summer and winter calving Holstein cows. J. Dairy Sci., 86(1): North Carolina State University, Raleigh, NC.
29. Bell, A., R. Slepetis and R. Ehrhardt, 1995. Growth and Accretion of Energy and Protein in the Gravid Uterus during Late Pregnancy in Holstein Cows. J. Dairy Sci., 78(9): 1954-1961.
30. Abeni, F., G. Bergoglio, G. Masoero, G.M. Terzano, M.C. Scatà, A. Borghese, A. Lucaroni and S. Ridolfi., 2001. Effetti della radiazione solare sul benessere di vacche di razza Valdostana al pascolo, pp: 155-164.
31. Accrosi, R.A., N. Govoni, R. Gaiani, C. Pezzi and E. Seren, 2005. Leptin, GH, PRL, insulin and metabolic parameters throughout the dry period and lactation in dairy cows. Rep. Dom. Anim., 40: 217-223.
32. Wheelock, J.B., R.P. Rhoads, M.J. VanBaale, S.R. Sanders and L.H. Baumgard, 2010. Effects of heat stress on energetic metabolism in lactate Holstein cows. J. Dairy Sci., 93: 644655.
33. Rollin, E., R.D. Berghaus, P. Rapnicki, S.M. Godden and M.W. Overton, 2010. The effect of injectable butaphosphan and cyanocobalamin on postpartum serum b Hydroxybutyrate, calcium and phosphorus concentrations in dairy cattle. J. Dairy Science, T., 93: 978-987.
34. Bertics, S., R. Grummer, C. Cadorniga-Valino and E. Stoddard, 1992. Effect of Prepartum Dry Matter Intake on Liver Triglyceride Concentration and Early Lactation. J. Dairy Sci., 75(7): 1914-1922.

35. Kessel, S., M. Stroehl, H. Meyer, S. Hiss, H. Sauerwein, F. Schwarz and R. Bruckmaie, 2008. Individual variability in physiological adaptation to metabolic stress during early lactation in dairy cows kept under equal conditions J. Anim. Sci., 86: 2903-2912.
36. Beever, D.E., 2006. The impact of controlled nutrition during the dry period on dairy cow health, fertility and performance. Anim. Reprod. Sci., 96: 212-226.
37. Re´mond, B., M. Cisse, A. Ollier and Y. Chilliard, 1991. Slow release somatotropin in dairy heifers and cows fed two levels of energy concentrate. Journal of Dairy Science, 74: 1370-1381.
38. Echternkamp, S.E., 1993. Relationship between placental development and calf birth weight in beef cattle. Anim. Reprod. Sci., 32: 1-13.
39. Zhang, W.C., T. Nakao, M. Moriyoshi, K. Nakada, T. Ohtaki, A.Y. Ribadu and Y. Tanaka., 1999. The relationship between plasma oestrone sulphate concentrations in pregnant dairy cattle and calf birth weight, calf viability, placental weight and placental expulsion. Anim. Reprod. Sci., 54: 169-178.
40. Guedon, L., J. Saumande and B. Desbals., 1999. Relationships between calf birth weight, prepartum concentrations of plasma energy metabolites and resumption of ovulation postpartum in Limousine suckled beef cows. J. Theriogenol. 1, 52(5): 779-789.
41. Karimi Sehkordi, M., M. Bolourchi, S. Safi and A. Rahimi Foroushani, 2012. Assocition between blood metabolites and pregnancy success at first service in Iraninan dairy cows. Global Vet., 9(6): 726-731.