The effect of ketones on estrogen, progesterone, ovulatory follicle size, estrus and fertility in Holstein Cows

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Abstract

This study aimed to investigate the effect of hyperketonemia on estrogen, progesterone and ovarian follicle size during postpartum as well as on pregnancy rate at first insemination. Regarding this, 90 Holstein cows were selected from one of the industrial dairy farms in Isfahan province, Iran. Estrus synchronization was routinely applied to all cows using the Ov Synch method. The blood sample of the cows was taken two times. The first blood sample was taken in 7 - 14 days after calving; to measure Beta hydroxybutyrate (BHBA), and the second sample was taken at the first insemination to measure the 17-β oestradiol and progesterone hormones. The pregnancy status was recorded at the first insemination. T-test analysis was used to evaluate the effects of hyperketonemia on the concentration of steroid hormones and pregnancy rate at first insemination. 4.4% of cows (4 of 90) had subclinical ketosis (SCK) (BHBA greater than 1000 μmol / L). Results showed that cows with BHBA levels greater than 600 μmol / L in 7 - 14 days of postpartum had lower 17-β oestradiol levels ($P = 0.04$) and higher progesterone levels ($P = 0.06$) at the time of insemination. Pregnant cows also had significantly lower serum BHBA and higher 17-β oestradiol levels than non-pregnant cows at first insemination. The levels of BHBA, estrogen, and progesterone were not significantly different in cows with different ovulation follicle sizes ($P > 0.05$). However, the level of BHBA was lower in cows that exhibit symptoms of estrus at the time of insemination ($P = 0.07$). 17-β oestradiol levels were higher and progesterone levels were lower in cows with estrus symptoms with $P = 0.07$ and $P = 0.01$, respectively. The present study confirms that higher ketone bodies have negative effects on 17-β oestradiol level and fertility. Also it appears that postpartum return to estrus is associated with high concentrations of 17-β oestradiol and a low concentration of BHBA in early lactation.

Keywords: Beta hydroxybutyrate (BHBA), Estrogen, Progesterone, Pregnancy at first insemination, Follicular size, Estrous Symptoms

1. Introduction

The dairy cows are subjected to a period of negative energy balance (NEB), metabolic stress, and body condition score (BCS) loss, due to the use of body reserves in response to excessive energy requirement for milk production in postpartum [1, 2]. The feeding rate is not sufficient to meet the requirements of milk production during this period. In addition, the genetic selection to produce more milk and an inappropriate feeding diet can increase the rate and duration of NEB [3, 4, 5]. In these circumstances, reduced blood glucose and hormonal changes; especially increased glucagon levels, lead to metabolic disorders such as glycogenolysis, lipolysis, beta-oxidation of fats, gluconeogenesis, and ketogenesis, and ultimately lead to the replacement of lipids and increasing the production of Ketones (such as acetic acid, acetone, and BHBA) in the liver [6].

The increase in the level of ketones during early lactation is due to the body's metabolic response to excess energy demand. However, the increased ketone levels in the blood are associated with poor fertility, decreased milk production, and risk of abomasum replacement [7]. Cattle with ketosis usually show symptoms of other diseases such as metritis, endometritis, placenta retention, and mastitis [8, 9, 10, 11].

It has been demonstrated that NEB affects subsequent fertility in early lactation by impaired hypothalamic-pituitary-ovarian axis [12]. The duration and amount of NEB are associated with high concentrations of growth hormone and low concentrations of insulin and IGF, which directly decreases the follicle quality and its response to gonadotropins [12, 13]. In addition, NEB is associated with a decrease in the concentration of LH surge, which in turn delays the onset of Corpus luteum activity, increases the prevalence of ovarian cyst, and reduces the chance of pregnancy at first insemination [5, 14]. Laminitis, high somatic cell count, low BCS, and increased milk production can have detrimental effects on the fertility of dairy cows and reduce the GnRH release. As a result, it reduces LH pulses and estradiol production and leads to impaired estrus behavior [15]. However, there are limited reports about the effects of NEB on postpartum estrus. It is assumed that NEB decreases estradiol concentration prior to ovulation which results in fewer estrus symptoms [13]. The aim of this study was to evaluate the effect of hyperketonemia on the concentrations of 17-β oestradiol (estradiol) and progesterone, ovulatory follicle size, and the estrus symptoms at insemination as well as pregnancy rate at first insemination.

2. Materials and Methods

2.1 The Study Sample

This study was conducted on 90 Holstein multiparous (2 - 5) cows in one of the dairy farms in Isfahan province. Cows were kept in the farmyard and fed based on TMR. The diet consisted of alfalfa, corn silage, and the concentrates containing barley, maize, soybeans, organic and mineral supplements, salts and micronutrients. Cows were milked by milking machine three times daily, at 6 a.m., 2 p.m., and 10 p.m. The average herd milk yield was 33 kg per cow per day.

2. 2 Sampling

The blood samples were taken during postpartum two times: first, 7 - 14 days of postpartum to measure the BHBA, and second; at first insemination, to measure estradiol and progesterone levels. 10 ml of blood was collected at each sampling time from the jugular vein. Serums transferred to the laboratory immediately and were stored at - 20 °C. The samples were recollected if they were hemolyzed.

2.3 Measurement of parameters in blood

Serums were finally sent to the clinical pathology laboratory under cold temperatures.

The BHBA content measured by Autoanalyzer BT-3000 (Biotechnica, Biotecnica Instruments, Italy) and Randox kit (Randox Laboratories Ltd, UK)). Also, estradiol content by ELISA with the sensitivity of 8.2 pg/ml, C.V.%: 3.9 and intra-assay and inter-assay coefficients of variations: 9 and 10 % (estradiol AccuBind, 4925-300, Monobind, USA), and progesterone content by ELISA with the sensitivity of 0.105 ng/ml, C.V.%: 3.9 as well as intra-assay and inter-assay coefficients of variations with 3.1 and 7 %, respectively (progesterone AccuBind, 4825-300, MonoBind, USA) were measured.

2.4 Management of Fertility

The Ov Synch (Ovulation Synchronization) protocol was performed for all cows after the clean test (32 to 35 days of postpartum) and confirmation of genital health. Ovulation follicular size and incidence of estrus symptoms were recorded on the day of insemination. Pregnancy diagnosed 32 to 35 days after insemination by Ultrasonography (easy-scan BCF model) and was confirmed 45 days after insemination. The date of calving, the date of the first insemination, the Subsequent possible insemination and pregnancy test results were recorded.

2.5 Statistical analysis

Data were analyzed using SPSS software, version 19. T-test statistical models were used to compare mean parameters (BHBA, estradiol, and progesterone) in two groups of cows with different BHBA values and also to compare mean parameters (estradiol and progesterone) in two groups of pregnant and non-pregnant cows at first insemination. Independent sample student t-test was used to compare the mean of the two groups with normal distribution and Mann-Whitney U-test was used for data that were not normal. The p-value of less than 0.05 or 0.1 was considered significant.

3. Results

3.1 The mean concentrations of BHBA, estradiol and progesterone

The mean concentrations of BHBA, estradiol, and progesterone are presented in **Table 1**.

3.2 The effect of hyperketonemia on the concentration of estrogen, progesterone and follicular size

In this study, cows were divided into two groups based on BHBA levels to evaluate the effect of hyperketonemia on estradiol and progesterone concentrations. BHBA levels of the first group were greater than or equal to 600 μmol / L and BHBA levels of the second group were less than 600 μmol / l. **Table 2** shows the effect of hyperketonemia on estradiol estradiol and progesterone concentrations. estradiol level in the first group was significantly lower than the second group (P = 0.04). No significant difference observed in the level of progesterone (P = 0.06) and follicular size (P = 0.1) between two groups.

3.3 The relationship between BHBA, estrogen, progesterone and ovarian follicle size with pregnancy

The cows also were divided into two groups of pregnant (n = 34) and non-pregnant (n = 56) at first insemination. The BHBA, estradiol, and progesterone values were statistically compared in the two groups and the results were presented in **Table 3**. According to the results, pregnant cows had lower BHBA and higher estradiol levels and the differences were statistically significant in both cases ($P < 0.05$). The mean of progesterone and ovarian follicle size were not significantly different between the pregnant and non-pregnant cows at first insemination.

3.4 The relationship between BHBA, estrogen, progesterone concentrations with follicular size

The cows were divided into two groups of follicular size equal or more than 1.7 cm (≥ 1.7) and follicular size less than 1.7 cm (< 1.7) at the time of insemination [16]. There were no significant differences in BHBA, estradiol, and progesterone concentrations between the two groups (**Table 4)**.

3.5 The relationship between the studied parameters on incidence of the estrus symptoms at insemination

The cows were divided into two groups based on exhibiting the estrus symptoms at the time of the first insemination. Group 1 (which showed estrus symptoms), compared with group 2 (which did not show estrus symptoms) had lower BHBA ($P = 0.07$), higher estradiol ($P = 0.07$), and lower progesterone levels ($P = 0.01$). Ovulatory follicle size was greater in the group that had estrus symptoms at insemination. Although, this difference was not significant ($P = 0.2$). There was no significant difference between the two groups in the rate of pregnancy at the first insemination (**Figure 1**).

4. Discussion

To the best of our knowledge, this is the first study to show the association between hyperketonemia and the level of steroid hormones, as well as the ovarian follicle size, and the appearance of estrus symptoms at insemination. Itle et al. [17] demonstrated the differences in estrus behaviors in cows with clinical ketosis compared to healthy cows one week before and at parturition. However, the effect of SCK on estrus behaviors was less noticeable. The prevalence of SCK in our study, by considering 1000 μmol / l threshold for BHBA, was 4.4% (4 of 90 cows), which is lower than the levels reported in the literature [14, 18, 19].

The higher prevalence is usually observed in high-producing dairy herds. These cows may have a greater genetic potential for high-milk production that can aggravate NEB and lead to SCK in early lactation. Several studies have established lower threshold levels for determination of SCK for blood BHBA or performed blood sampling during the first week after calving. In these cases, the prevalence of SCK was higher [7, 14, 19]. In our study, the prevalence of SCK was 24.4% (22 of 90 cows) by considering 600 μmol BHBA threshold levels.

A dairy cow can get SCK and recover within five days [14, 20]. In the present study, the cows were tested only once which was between 7 and 14 days of postpartum. In a study by McArt et al. [14], the SCK prevalence peak was reported within the five days of lactation (DMI), whereas in our study, the sampling was performed after seven days of postpartum. Nevertheless, our study was performed according to the methods in previous literature [21, 22].

In ideal conditions, the sampling should perform several times during the first month of lactation to reduce misclassification bias. Unfortunately, there were limitations regarding the time and budget in our study. However, the present study followed the same sampling protocol which also used in a large number of studies that examined SCK risk factors [5, 22, 23].

4.1 The Relationship between Steroid Hormones and Hyperketonemia

The cows also were divided into two groups based on BHBA levels with equal to or greater than 600 μmol / L (n = 22) and less than 600 μmol / L (n = 68). Cows with BHBA greater than 600 μmol / L at 7 - 14 days postpartum were found to have lower estradiol (P = 0.04) and higher progesterone (P = 0.06) at insemination. In general, cows with NEB (Hyperketonemia), elevated BHBA, NEFA, and urea in their blood which causes changes in the follicular environment, oocyte quality, and ultimately a defect in oocyte development. On the other hand, the NEB affects the liver which decreases insulin-like growth factor that plays as gonadotropin in the ovary. Reduction in this factor decreases the follicular growth and estradiol synthesis [24]. The follicles are the source of estradiol. The follicles that are not properly grown, produce less estradiol. Therefore, cows with Hyperketonemia may have lower estradiol levels. Moreover, an increase in estradiol can also result in the loss of corpus luteum and the decrease in progesterone [25]. Comin et al. [26] investigated the effect of energy deficiency on cows that were synchronized. The energy decrease reduced the estradiol levels in the active follicles and the ratio of estradiol to progesterone. This was due to the negative effect of increased non-esterified fatty acids (NEFA) and the type II and III proteins that bound to insulin-like growth factor. These factors can contribute to the incomplete Luteinization of the larger follicle and the lack of ovulation through the inability of estradiol.

4.2 The relationship between the Steroid Hormones with Pregnancy at First insemination

The relationship between estradiol and progesterone with pregnancy at first insemination was also investigated. According to the results, the cows that were conceived during the first insemination had significantly higher estradiol (52.19 ± 3.7) compared to the non-pregnant cows (49.12 ± 4.8).

One explanation is that in cows with Hyperketonemia, the follicular growth has reduced which was due to the depletion of insulin-like growth factor and eventually decreasing the estradiol synthesis and LH rate which result in delayed ovulation and lowering the fertility. In agreement with our study, Boland et al. [27] stated that the dietary energy depletion (a condition similar to NEB and an increase in ketones) could lead to follicle growth depletion and damage, and ultimately reduction in estradiol production. As a result, the time of the first ovulation after calving is longer so as the time of calving to the next pregnancy.

4.3 Relationship between Hyperketonemia and pregnancy at first insemination

Most cows have a negative energy balance during early lactation, using fat and protein to compensate. In addition to the lack of growth of the primary follicles, this action increases the blood urea and BHBA, which delay the onset of the ovarian cycle and impaired fertility [28]. Metabolism of proteins in the body following reduction or increase in diet protein increases blood urea, which can reduce fertility [29]. High dairy-producing cows usually have low fertility rates, which could be because of early fetal deaths due to NEB and the increased BHBA, NEFA and decreased blood glucose [30].

The result of the present study is consistence with other studies that show the negative effects of SCK on fertility efficiency [5, 9, 12, 14]. Several studies in north of USA used the ovulation synchronization protocol and showed the effects of SCK during the first 30 days of lactation on fertility parameters (including the likelihood of pregnancy in first insemination and the possibility of removing the cow from the herd) [5, 14, 31]. However, some studies have indicated the association between SCK in early lactation and low fertility efficiency without considering the synchronization protocols [9, 32, 33].

The present study was conducted based on Ov synch protocol and the cows that became pregnant during first insemination had lower BHBA concentration (0.38 ± 0.15 compared to 0.54 ± 0.26; $P = 0.008$). These are in line with the findings of Reist et al. [32]. They showed that the interval between calving to the first insemination was 10 days longer in cows with NEB and these cows also have higher blood ketone concentration.

Ospina et al. [5] showed that the probability of pregnancy in cows with SCK is lower. Walsh et al. [9] reported that the probability of pregnancy at first insemination in cows with SCK was 20 - 50% lower than other cows. These findings are consistent with the results of our study. Previous studies have highlighted that the decrease in fertility efficiency is attributed to the delayed return to cyclicity related to the reduced GnRH and LH pulses, which is necessary for the follicle development and ovulation [12].

4.4 Relationship of Hyperketonemia with Ovarian Follicle Size

As mentioned above, the increase in ketone concentration by lowering the insulin-like growth factor results in follicular growth depletion and ultimately decrease in estradiol production. It can be expected that hyperketonic cows have smaller follicles which were also observed in our results. The cows that had smaller ovulatory follicles at insemination (less than 1.7 cm) than the other group (greater than 1.7 cm) had higher BHBA values. Although it was not statistically significant (0.51 ± 0.17 compared to 0.46 ± 0.26; $P = 0.1$). Bergfeld et al [34] investigated the effect of energy levels on the growth and development of ovarian follicles in heifers. According to their result, heifers that were fed with a higher-energy diet had larger follicles and more estradiol at insemination. In agreement with our study, Wathes et al. [35] also stated that energy deficiency causes some alternations in the liver which increases the proteins bound to insulin-like growth factor and eventually decreases the concentrations of growth hormone receptors and insulin-like growth factor.

Therefore, a decrease in the concentration of insulin-like growth factors damages follicles and decreases the production of estrogen.

4.5 The Relationship between Hyperketonemia and Steroid Hormones with the Incidence of Estrus symptoms

According to the results cows that showed symptoms of estrus at insemination had lower BHBA (0.43 ± 0.23 compared to 0.54 ± 0.20; P = 0.07) and higher estradiol (51.4 ± 3.9 compared to 48.9 ± 5.1; P = 0.07). Also, in these cows the progesterone levels were significantly lower than the cows with no estrus symptoms (0.67 ± 0.17 compared to 0.84 ± 0.25; P= 0.01). Hyperketonemia by decreasing the estradiol caused a delay in ovulation and a decrease in the incidence of estrus symptoms. On the other hand, estradiol depletion prevented symptoms of estrus by damaging the corpus luteum and reduced the production of progesterone. In addition, results of similar research showed that dietary restriction decreased estrus behavior by reducing central nervous system sensitivity to estradiol and decreasing the number of estradiol receptors in the brain. Research has demonstrated that these abnormalities can be treated by increasing dietary energy [36].

4.6 The Relationship between milk production and BCS with the hyperketonemia and pregnancy rate

In the present study, it was found that cows with higher milk production and lower BCS had higher serum BHBA levels ($p \leq 0.05$) and lower FSCR ($P > 0.05$). Similarly, Esmaili and Mirzaei (2015) showed that the pregnancy rate was significantly higher in cows with a

BCS above 3 during the 35 - 40 days after calving than in cows with a BCS of 3 and less than 3 [37]. Also, Barletta et al (2017), reported that the cows with low BCS had significantly higher serum levels of NEFA and BHBA than those with higher BCS, which also harmed reproduction [38].

5. Conclusion

The present study confirms the long negative effects of SCK on fertility efficiency. Pregnancy rates at first insemination as well as estradiol concentrations were lower in hyperketonic cows. In addition, the cows that show estrus symptoms at insemination had lower BHBA, higher estradiol, and lower progesterone concentrations. Also, the cows with follicle size greater than 1.7 cm at insemination had lower BHBA than the cows with smaller follicles, although this difference was not statistically significant. According to the results, it is recommended that the measurement of ketones before and after calving should be one of the routine functions in dairy herds so that early SCK treatments could improve future fertility and reproductive health of dairy herds.

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References

1. Drackley JK. Biology of dairy cows during the transition period: The final frontier?. Journal of dairy science. 1999 Nov 1; 82 (11): 2259-2273. doi: 10.3168/jds.S0022-0302(99)75474-3
2. Herdt TH. Ruminant adaptation to negative energy balance: Influences on the etiology of ketosis and fatty liver. Veterinary Clinics: Food Animal Practice. 2000 Jul 1; 16 (2): 215-230. doi: 10.1016/S0749-0720(15)30102-X
3. Van Arendonk JA, Nieuwhof GJ, Vos H, Korver S. Genetic aspects of feed intake and efficiency in lactating dairy heifers. Livestock Production Science. 1991 Dec 1; 29 (4): 263-275. doi: 10.1016/0301-6226(91)90103-W
4. Dechow CD, Rogers GW, Klei L, Lawlor TJ. Heritabilities and correlations among body condition score, dairy form and selected linear type traits. Journal of dairy science. 2003 Jun 1; 86 (6): 2236-2242. doi: 10.3168/jds.S0022-0302(03)73814-4
5. Ospina PA, Nydam DV, Stokol T, Overton TR. Associations of elevated nonesterified fatty acids and β-hydroxybutyrate concentrations with early lactation reproductive performance and milk production in transition dairy cattle in the northeastern United States. Journal of dairy science. 2010 Apr 1; 93 (4): 1596-1603. doi: 10.3168/jds.2009-2852
6. Epperson WB. Risk factors for metabolic disease. Tri-State Dairy Nutrition Conference, 14: 2005. pp. 31-35
7. McArt JA, Nydam DV, Oetzel GR, Overton TR, Ospina PA. Elevated non-esterified fatty acids and β-hydroxybutyrate and their association with transition dairy cow performance. The Veterinary Journal. 2013 Dec 1; 198 (3): 560-570. doi: 10.1016/j.tvjl.2013.08.011
8. Roche JF. The effect of nutritional management of the dairy cow on reproductive efficiency. Animal reproduction science. 2006 Dec 1; 96 (3-4): 282-296. doi: 10.1016/j.anireprosci.2006.08.007
9. Walsh RB, Walton JS, Kelton DF, LeBlanc SJ, Leslie KE et al. The effect of subclinical ketosis in early lactation on reproductive performance of postpartum dairy cows. Journal of dairy science. 2007 Jun 1; 90 (6): 2788-2796. doi: 10.3168/jds.2006-560
10. Mellado M, García JE, Véliz Deras FG, de Santiago MD, Mellado J et al. The effects of periparturient events, mastitis, lameness and ketosis on reproductive performance of Holstein cows in a hot environment. Austral journal of veterinary sciences. 2018 Jan; 50 (1): 1-8. Doi: 10.4067/S0719-81322018000100102
11. Garro CJ, Mian L, Cobos Roldán M. Subclinical ketosis in dairy cows: prevalence and risk factors in grazing production system. Journal of animal physiology and animal nutrition. 2014 Oct; 98 (5): 838-844. doi: 10.1111/jpn.12141
12. Butler WR. Energy balance relationships with follicular development, ovulation and fertility in postpartum dairy cows. Livestock production science. 2003 Oct 1; 83 (2-3): 211-8. doi: 10.1016/S0301-6226(03)00112-X
13. Lucy MC. Regulation of ovarian follicular growth by somatotropin and insulin-like growth factors in cattle. Journal of Dairy Science. 2000 Jul 1; 83 (7): 1635-1647. doi: 10.3168/jds.S0022-0302(00)75032-6

14. McArt JA, Nydam DV, Oetzel GR. Epidemiology of subclinical ketosis in early lactation dairy cattle. Journal of dairy science. 2012 Sep 1; 95 (9): 5056-5066. doi: 10.3168/jds.2012-5443
15. Dobson H, Smith RF, Royal MD, Knight CH, Sheldon IM. The high- producing dairy cow and its reproductive performance. Reproduction in domestic animals. 2007 Sep; 42: 17-23. doi: 10.1111/j.1439-0531.2007.00906.x
16. Ginther, O. The theory of follicle selection in cattle. Domestic animal endocrinology. 2016 57: 85-99.
17. Itle AJ, Huzzey JM, Weary DM, Von Keyserlingk MA. Clinical ketosis and standing behavior in transition cows. Journal of dairy science. 2015 Jan 1; 98 (1): 128-134. doi: 10.3168/jds.2014-7932
18. Oetzel GR. Monitoring and testing dairy herds for metabolic disease. Veterinary Clinics: Food Animal Practice. 2004 Nov 1; 20 (3): 651-674. doi: 10.1016/j.cvfa.2004.06.006
19. Macrae AI, Burrough E, Forrest J. Assessment of nutrition in dairy herds: use of metabolic profiles. Cattle Practice. 2012; 20 (2): 120-127.
20. Valergakis EG, Oikonomou G, Arsenos G, Banos G. Epidemiologic characteristics of subclinical ketosis in dairy cows. In Proceedings: XXVIIth World Buiatrics Congress, Lisbon 2012.
21. Berge AC, Vertenten G. A field study to determine the prevalence, dairy herd management systems, and fresh cow clinical conditions associated with ketosis in western European dairy herds. Journal of dairy science. 2014; Apr 1; 97 (4): 2145-2154. doi: 10.3168/jds.2013-7163
22. Vanholder T, Papen J, Bemers R, Vertenten G, Berge AC. Risk factors for subclinical and clinical ketosis and association with production parameters in dairy cows in the Netherlands. Journal of dairy science. 2015 Feb 1; 98 (2): 880-888. doi: 10.3168/jds.2014-8362
23. Suthar VS, Canelas-Raposo J, Deniz A, Heuwieser W. Prevalence of subclinical ketosis and relationships with postpartum diseases in European dairy cows. Journal of dairy science. 2013 May 1; 96 (5): 2925-2938. doi: 10.3168/jds.2012-6035
24. Kendrick KW, Bailey TL, Garst AS, Pryor AW, Ahmadzadeh A et al. Effects of energy balance on hormones, ovarian activity, and recovered oocytes in lactating Holstein cows using transvaginal follicular aspiration. Journal of dairy science. 1999 Aug 1; 82 (8): 1731-1741. doi: 10.3168/jds.S0022-0302(99)75403-2
25. Souza AH, Gümen A, Silva EP, Cunha AP, Guenther JN et al. Supplementation with estradiol-17β before the last gonadotropin-releasing hormone injection of the Ovsynch protocol in lactating dairy cows. Journal of dairy science. 2007 Oct 1; 90 (10): 4623-4634. doi: 10.3168/jds.2007-0172
26. Comin A, Gerin D, Cappa A, Marchi V, Renaville R et al. The effect of an acute energy deficit on the hormone profile of dominant follicles in dairy cows. Theriogenology. 2002 Sep 1; 58 (5): 899-910. doi: 10.1016/S0093-691X(02)00922-6
27. Boland MP, Lonergan P, O'callaghan D. Effect of nutrition on endocrine parameters, ovarian physiology, and oocyte and embryo development. Theriogenology. 2001 Apr 1; 55(6): 1323-1340. doi: 10.1016/S0093-691X(01)00485-X

28. Hojman D, Kroll O, Adin G, Gips M, Hanochi B et al. Relationships between milk urea and production, nutrition, and fertility traits in Israeli dairy herds. Journal of Dairy Science. 2004 Apr 1; 87 (4): 1001-1011. doi: 10.3168/jds.S0022-0302(04)73245-2

29. Garcia-Bojalil CM, Staples CR, Risco CA, Savio JD, Thatcher WW. Protein degradability and calcium salts of long-chain fatty acids in the diets of lactating dairy cows: productive responses. Journal of Dairy Science. 1998 May 1; 81 (5): 1374-1384. doi: 10.3168/jds.S0022-0302(98)75701-7

30. Rhoads ML, Rhoads RP, Gilbert RO, Toole R, Butler WR. Detrimental effects of high plasma urea nitrogen levels on viability of embryos from lactating dairy cows. Animal reproduction science. 2006 Jan 1; 91 (1-2): 1-0. doi: 10.1016/j.anireprosci.2005.02.009

31. Chapinal N, LeBlanc SJ, Carson ME, Leslie KE, Godden S et al. Herd-level association of serum metabolites in the transition period with disease, milk production, and early lactation reproductive performance. Journal of dairy science. 2012 Oct 1; 95 (10): 5676-5682. doi: 10.3168/jds.2011-5132

32. Reist M, Koller A, Busato A, Kupfer U, Blum JW. First ovulation and ketone body status in the early postpartum period of dairy cows. Theriogenology. 2000 Sep 15; 54 (5): 685-701. doi: 10.1016/S0093-691X(00)00383-6

33. Cook NB, Ward WR, Dobson H. Concentrations of ketones in milk in early lactation, and reproductive performance of dairy cows. Veterinary Record. 2001 Jun 23; 148 (25): 769-772. doi: 10.1136/vr.148.25.769

34. Bergfeld EG, Kojima FN, Cupp AS, Wehrman ME, Peters KE et al. Ovarian follicular development in prepubertal heifers is influenced by level of dietary energy intake. Biology of reproduction. 1994; Nov 1; 51 (5): 1051-1057. doi: 10.1095/biolreprod51.5.1051

35. Wathes DC, Fenwick M, Cheng Z, Bourne N, Llewellyn S et al. Influence of negative energy balance on cyclicity and fertility in the high producing dairy cow. Theriogenology. 2007 Sep 1; 68: S232-241. doi: 10.1016/j.theriogenology.2007.04.006

36. Zamiri M. Reprodoction physiology. Rasht, Haghshenas Publication: 448; 2006

37. Esmaili Tazangi M.K, and Mirzaei A. The effect of body condition loss and milk yield on the efficiency of Ovsynch in cycling Holstein dairy cows. Revue de Médecine Vétérinaire. 2015; 166: 345-349.

38. Barletta RV, Maturana Filho M, Carvalho PD, Del Valle TA, Netto AS et al. Association of changes among body condition score during the transition period with NEFA and BHBA concentrations, milk production, fertility, and health of Holstein cows. Theriogenology. 2017; 104: 30-36.

Table 1. Mean concentration of different parameters in the studied cows (n = 90)

Parameters	Mean ± SD
BHBA (µmol/L)	480 ± 0.23
Eestradiol (pg)	50.2 ± 4.6
Progesterone (ng)	0.75 ± 0.22

Table 2. Mean of estrogen, progesterone and follicle size in cows with different BHBA values

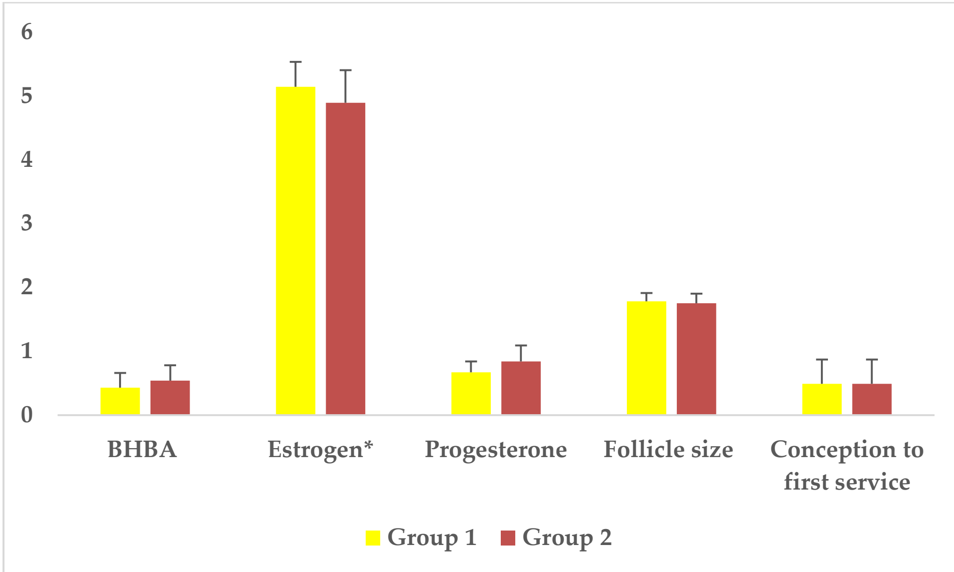
BHBA (µmol/L)	< 600 (n = 68)	≥ 600 (n = 22)	P - value
Eestradiol (pg)	51.16 ± 4.3	47.55 ± 4.9	0.04
Progesterone (ng)	0.72 ± 0.23	0.85 ± 0.16	0.06
Follicle size (cm)	1.7 ± 0.15	1.7 ± 0.08	0.1
BCS 30 days after calving	2.9 ± 0.22	2.5 ± 0.29	0.001
Milk production (Kg per cow per day)	28.52 ± 2.2	33.63 ± 4.2	0

Table 3. Mean concentrations of BHBA, estrogen, progesterone and follicle size in pregnant and non-pregnant cows at first insemination

Parameters	Conceived (n = 34)	Non-conceived (n = 56)	P - Value
BHBA (µmol/L)	380 ± 0.15	540 ± 0.26	0.008
Eestradiol (pg)	52.19 ± 3.7	49.12 ± 4.8	0.02
Progesterone (ng)	0.73 ± 0.19	0.76 ± 0.25	0.5
Follicle size (cm)	1.76 ± 0.1	1.76 ± 0.16	0.5
BCS 30 days after calving	2.91 ± 0.32	2.85 ± 0.26	0.6
Milk production (Kg per cow per day)	29.57 ± 3.5	30.11 ± 3.7	0.5

Table 4. Mean concentration of BHBA, 17-β oestradiol and progesterone in cows with different follicular size

Follicle size (cm)	< 1.7 cm (n = 39)	≥ 1.7 cm (n = 58)	P - value
BHBA (μmol/L)	0.51 ± 0.17	0.46 ± 0.26	0.1
Eestradiol (pg)	50.04 ± 5.3	50.4 ± 4.4	0.8
Progesterone (ng)	0.76 ± 0.24	0.75 ± 0.23	0.8



* Estrogen/10

** BHBA (µmol/L), estradiol (pg), Progesterone (ng), Follicle size (cm)

Figure 1. Mean concentration of studied parameters based on incidence (group 1) or lack of estrus symptoms (group 2) at insemination time