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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 Ovsynch 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 nonpregnant cows at first insemination. The levels of BHBA, oestradiol, 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.

Key words: 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 [3–5]. 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 [8–11].

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

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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. 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 (Biotecnica Instruments, Rome, Italy) and Randox kit (Randox Laboratories Ltd, Crumlin, UK). Also, estradiol content by ELISA with the sensitivity of 8.2 pg/mL, C.V.%: 3.9 and intraassay and interassay coefficients of variations: 9% and 10% (estradiol AccuBind, 4925-300, Monobind, Lake Forest, CA, USA), and progesterone content by ELISA with the sensitivity of 0.105 ng/mL, C.V.%: 3.9 as well as intraassay and interassay coefficients of variations with 3.1% and 7%, respectively (progesterone AccuBind, 4825-300, Monobind) were measured.

2.4. Management of fertility

The Ovsynch (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 (IBM Corporation, Armonk, NY, USA). 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 nonpregnant 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. Mean concentrations of BHBA, estradiol and progesterone

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

3.2. 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

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

Parameters	Mean ± SD
BHBA (μmol/L)	480 ± 0.23
Estradiol (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
Estradiol (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

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. Relationship between BHBA, Estradiol, progesterone and ovarian follicle size with pregnancy

The cows also were divided into two groups of pregnant (n = 34) and nonpregnant (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 nonpregnant cows at first insemination.

3.4. 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. 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).

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

Parameters	Conceived (n = 34)	Nonconceived (n = 56)	p-value
BHBA (µmol/L)	380 ± 0.15	540 ± 0.26	0.008
Estradiol (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)	510 ± 0.17	460 ± 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

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].

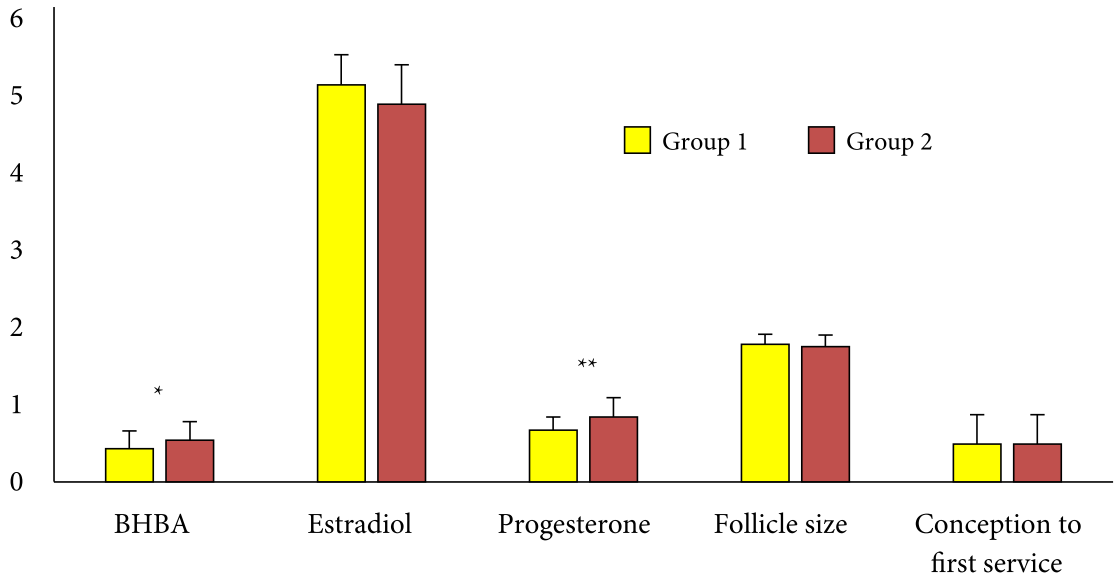

Figure. Mean concentration of studied parameters based on incidence (group 1) or lack of estrus symptoms (group 2) at insemination time this sentence followed by BHBA (mmol/L=1000 µmol/L), Estradiol (pg), Progesterone (ng), Follicle size (cm). * p<0.1 and ** p<0.05

4.1. 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 nonesterified 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. Relationship between 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 pg) compared to the nonpregnant cows (49.12 ± 4.8 pg).

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 Ovsynch protocol and the cows that became pregnant during first insemination had lower BHBA μmol/L concentration (380 ± 0.15 compared to 540 ± 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 μmol/L 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 decreases 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 μmol/L. Although it was not statistically μmol/L significant (510 ± 0.17 compared to 460 ± 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. 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 mmol/L compared to 0.54 ± 0.20 mmol/L; p = 0.07) and higher estradiol (51.4 ± 3.9 pg compared to 48.9 ± 5.1 pg; p = 0.07). Also, in these cows the progesterone levels were significantly lower than the cows with no estrus symptoms (0.67 ± 0.17 ng compared to 0.84 ± 0.25 ng; 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. 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 ≤ 0.05) and lower conception at first service (p > 0.05). Similarly, Esmaili Tazangi 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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