

# Veterinary and Comparative Biomedical Research

## ORIGINAL ARTICLE

### Investigation of the Changes in NEFA during the Transition Period and its Relationship with Reproductive Performance in Holstein Dairy Cows

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Changes in NEFA during the Transition Period and Reproductive Performance

#### Abstract

This study investigate the changes in serum non-esterified fatty acids (NEFA) during the transition period and to evaluate their relationship with the reproductive performance of Holstein dairy cows. Thirty multiparous Holstein cows (parities 2–5) from a large industrial dairy herd in Shahrekord were enrolled during the last week of the dry period. Blood samples were collected at 10 and 30 days after calving. Samples were centrifuged at 1700 × g for 15 minutes, and serum was separated and stored at -20°C until analysis. Serum NEFA concentrations were determined using commercial Randox kits (UK). Reproductive data, including calving date, date of first insemination, number of inseminations, and pregnancy outcomes, were recorded. Statistical analyses were performed using SPSS (version 19). Serum NEFA concentrations increased significantly ( $P < 0.05$ ) after calving compared with the prepartum period, reaching the highest value at 10 days post-calving and declining thereafter. Mean NEFA levels at one month postpartum were significantly higher in cows that failed to conceive after the first insemination compared to cows that became pregnant ( $P < 0.05$ ). At 10 days postpartum, NEFA concentrations were significantly lower ( $P < 0.05$ ) in cows that received their first insemination before 75 days compared to those inseminated later, suggesting that elevated NEFA was associated with a prolonged calving-to-first-insemination interval. When cows were categorized by calving-to-conception interval (<80 days, 80–150 days, >150 days), the lowest NEFA concentrations were observed in the <80-day group, although differences were not statistically significant ( $P > 0.05$ ). Similarly, cows conceiving with fewer than three inseminations generally exhibited lower NEFA values. The findings confirm that dairy cows experience negative energy balance during the transition period, reflected by elevated NEFA concentrations. Monitoring NEFA dynamics during this period provides a useful indicator for predicting and improving reproductive performance in dairy herds.

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## Introduction

The transition period in dairy cows, encompassing three weeks before to three weeks after parturition, is considered the most critical phase in the reproductive and physiological cycle of these animals. During this period, a state known as negative energy balance (NEB) occurs due to reduced dry matter intake and significantly increased energy demands for fetal growth and the initiation of lactation. A direct consequence of NEB is the enhanced mobilization of body fat reserves and the release of non-esterified fatty acids (NEFA) into the bloodstream, making NEFA concentration a key indicator for assessing the metabolic status of dairy cows (1).

Historically, classical studies have reported a negative correlation between increased milk yield and reduced reproductive efficiency (2). This perspective led to the notion of an inherent and inevitable trade-off between milk production and fertility. However, subsequent research has challenged this interpretation, demonstrating that this relationship is not absolute and can be modulated through optimal management, particularly in the realm of nutrition (3, 4). This paradox indicates that reduced fertility in high-producing cows is not necessarily a direct result of high milk yield per se, but is more influenced by the associated metabolic changes, especially NEB and elevated NEFA concentrations. In other words, increased NEFA levels appear to have a direct and detrimental effect on reproductive efficiency.

Emerging evidence supports this hypothesis. For instance, Kia et al. (2023) showed that elevated prepartum NEFA concentrations are associated with the occurrence of postpartum diseases and a decline in fertility indices (5). Similarly, Wang et al. (2022) reported that the severity of NEB and increased NEFA in early lactation are correlated with reduced milk production and delayed return to estrous activity (6). An influential study by Ospina et al. (2010) quantitatively demonstrated that cows with high postpartum concentrations of NEFA and BHBA experienced a 16% and 13% reduction, respectively, in the likelihood of pregnancy by 70 days post-calving (7). Furthermore, other factors such as body condition score (BCS) play a role in this relationship. Mirzaei et al. (2023) reported that loss of body condition in early lactation is associated with increased NEFA, disruption of the metabolic hormone axis (e.g., IGF-I), and ultimately, reduced fertility (8). A recent study by Luke et al. (2024) also emphasized that assessing the relationship between NEFA and fertility must account for milk production level and other health indicators, as metabolic changes play a more determining role than high milk yield itself (9).

Despite this evidence, most research has focused on NEFA levels within a limited timeframe (prepartum or a few weeks postpartum) and less on the trajectory of NEFA changes from the dry period through postpartum and its association with precise fertility indices.

Moreover, the majority of previous studies have been conducted under the management practices and climatic conditions of Europe, America, or Australia. However, limited information is available regarding NEFA levels and reproductive performance in Holstein dairy cows under the specific management and climatic conditions of Iran. Given that climate and local management may influence energy balance and reproductive outcomes, our study aims to fill this knowledge gap for Iranian dairy herds.

Therefore, the primary objective of the present study is to investigate the pattern of NEFA changes throughout the transition period and evaluate its association with precise reproductive indices in Holstein dairy cows in Chaharmahal and Bakhtiari Province. The results of this research could provide a better understanding of the role of NEFA as a prognostic metabolic indicator for managing fertility in dairy herds under Iranian climatic conditions.

## Materials and Methods

### Experimental Animals and Management

This study was conducted on 30 multiparous Holstein dairy cows (2 to 5 parities) from a large commercial dairy farm in Shahrekord County, Chaharmahal and Bakhtiari Province, Iran (32°19'32" N latitude, 50°51'52" E longitude). The average temperature during the study period ranged from 5 °C to 25 °C.

All cows were enrolled during the last week of the dry period. Blood samples were randomly collected approximately one week before the expected calving date, as estimated by the farm management. After calving, blood samples were collected from each cow at 10 and 30 days postpartum. A total of 30 cows that calved within the expected timeframe were included in the study, while cows that calved more than one week earlier or later than predicted were excluded. Subsequent sampling continued with these 30 cows; however, three animals were later removed during the study period due to various reasons at the discretion of the farm management.

All animals were housed in open-sided barns in the same husbandry unit and received a total mixed ration (TMR) ad libitum, which included alfalfa hay, corn silage, and a concentrate mixture containing corn, soybean meal, and bone meal. The cows were milked three times daily at 6:00 AM, 2:00 PM, and 10:00 PM using a milking parlor system. The average herd milk production was 33 kg per cow per

day. The detailed composition of the total mixed ration (TMR) and the concentrate portion for the last week of pre-

calving, 10 days postpartum, and one month postpartum are presented in Tables 1 and 2, respectively.

**Table 1.** Composition of the total mixed ration (TMR) for the Holstein dairy herd during different stages of the transition period. Values are expressed in kg per cow per day.

Diet Ingredient	Time		
	One week pre-calving (Precalving ration) (kg)	10 days post-calving (Fresh cow) (kg)	One-month post-calving (milky cow) (kg)
<b>Concentrate</b>	7	12	19.7
Alfalfa	2.7	3	2.6
Corn silage	18	18	36.5
Wet pomace	0	4	4
Straw	0.5	1	1.8
Water	3	1.5	3.5

**Table 2.** Composition of the concentrate portion of the diet for Holstein dairy cows during different stages of the transition period. Values are expressed in grams per cow per day.

Diet Ingredient	Time	
	One week pre-calving (Precalving ration) (gr)	10 days post-calving and One month post-calving (milking cow ration) (gr)
<b>Corn</b>	300	360
<b>Barley</b>	250	200
<b>Soybean meal</b>	160	190
<b>Wheat bran</b>	128.5	49.5
<b>Meat powder</b>	40	70
<b>Full-fat soybean</b>	30	40
<b>Sodium bicarbonate</b>	15	15
<b>Pure fat powder</b>	0	25
<b>Sodium sesquicarbonate</b>	0	5
<b>Calcium carbonate</b>	20	8
<b>Magnesium oxide</b>	0	3
<b>Dicalcium phosphate</b>	0	3
<b>Salt</b>	0	4
<b>Premix (mineral-vitamin supplement)</b>	20	15
Benzoplex chelate	0.5	0.5
Bentonite	5	10
Slow-release urea	0	0
Toxin binder	3	2
Calcium chloride	10	0
Ammonium chloride	5	0
Magnesium sulfate	13	0
<b>Total</b>	<b>1000</b>	<b>1000</b>

### Blood Sampling and Analysis

Blood samples were collected from the coccygeal vein into 10-mL vacuum tubes without anticoagulant. Following collection, samples were allowed to clot at room temperature for 30 minutes and subsequently centrifuged at  $1700 \times g$  for 15 minutes at  $4^{\circ}C$  to separate serum. The obtained serum was aliquoted and stored at  $-20^{\circ}C$  until analysis.

Serum concentrations of non-esterified fatty acids (NEFA) were determined using a commercial enzymatic

colorimetric assay kit (Randox Laboratories Ltd., UK; Cat. No. FA 115), according to the manufacturer's instructions. The assay principle is based on the enzymatic conversion of NEFA to acyl-CoA synthetase, acyl-CoA oxidase, and peroxidase, resulting in a colorimetric product measured spectrophotometrically at 546 nm. The analytical sensitivity of the assay was 0.001 mmol/L, and both intra- and inter-assay coefficients of variation were  $<5\%$ .

This methodology aligns with established protocols for assessing metabolic status in transition dairy cows, as described in previous studies (7).

### Reproductive Management and Pregnancy Diagnosis

Following clinical examination (performed one month postpartum) to confirm reproductive tract health and the completion of a voluntary waiting period (50 days postpartum), all cows underwent an Ovsynch protocol for synchronization of ovulation. The Ovsynch protocol was administered as follows: intramuscular injection of 100 µg GnRH (Gonadorelin) on day 0, followed by 25 mg PGF2α (Dinoprost tromethamine) on day 7, and a second injection of 100 µg GnRH 56 hours after PGF2α administration. Timed artificial insemination (TAI) was performed 16-20 hours after the final GnRH injection.

Pregnancy diagnosis was conducted via transrectal ultrasonography 30-35 days post-insemination using a portable ultrasound scanner equipped with a 7.5 MHz linear-array transducer. Pregnancy status was reconfirmed by rectal palpation 45 days post-insemination. Calving dates, dates of first and subsequent inseminations, and pregnancy test results were systematically recorded for all animals.

### Reproductive Performance Assessment and Group Classification

Reproductive performance was evaluated based on four key parameters: 1) conception rate at first artificial insemination (AI), 2) calving to first insemination interval (CFI), 3) calving to conception interval (CCI), and 4) number of inseminations per conception (NIPC).

For conception rate analysis, cows were classified into pregnant (n = 9) and non-pregnant (n = 21) groups following the first postpartum insemination. The CFI was analyzed by categorizing animals into two groups: early-inseminated (CFI ≤ 75 days; n = 24) and late-inseminated (CFI > 75

days; n = 6). Based on CCI, cows were classified into three categories: short (CCI < 80 days; n = 13), intermediate (CCI = 80-150 days; n = 5), and extended (CCI > 150 days; n = 12) intervals. For NIPC analysis, animals were grouped as requiring ≤ 3 inseminations (n = 23) or > 3 inseminations (n = 7) to achieve conception.

### Statistical Analysis

Serum NEFA concentrations were compared among different reproductive performance groups using appropriate statistical methods. An independent samples t-test was employed for comparisons between two groups (conception rate at first AI, CFI, and NIPC categories), while one-way analysis of variance (ANOVA) followed by Tukey's post-hoc test was used for comparisons among three CCI groups. Normality of data distribution was confirmed using the Shapiro-Wilk test, and homogeneity of variances was verified with Levene's test. Non-parametric alternatives (Mann-Whitney U test for two groups and Kruskal-Wallis test for multiple groups) were utilized when assumptions of parametric tests were violated. All statistical analyses were performed using SPSS version 19.0 (IBM Corporation, Armonk, NY, USA), with statistical significance set at P < 0.05.

## Results

### Changes in Serum NEFA Concentrations

Table 3 presents the changes in serum non-esterified fatty acid (NEFA) concentrations in all study animals. Serum NEFA concentration increased significantly (p < 0.05) after calving compared to the end of the dry period, reaching its peak at 10 days postpartum. Subsequently, NEFA levels decreased progressively with increasing time from calving, returning to their minimum levels by one month postpartum.

**Table 3.** Mean serum NEFA concentrations at different time points around parturition

Time relative to parturition	NEFA (µmol/L)
One week pre-calving	347.4 ± 23.6 <sup>a</sup>
10 days post-calving	678.9 ± 48.2 <sup>b</sup>
One-month post-calving	319.1 ± 11.6 <sup>a</sup>

Different superscript letters within a column denote statistically significant differences (p < 0.05).

### Comparison of Mean Metabolite Concentrations in Cows with Different Reproductive Performance

#### Pregnancy at First Artificial Insemination

Among the 30 cows included in the study, 9 animals conceived following the first postpartum insemination,

resulting in a first-insemination pregnancy rate of 30%. Table 4 presents the comparative analysis of serum NEFA concentrations between pregnant and non-pregnant cows at different sampling time points around parturition. The mean serum NEFA concentration at one month postpartum was significantly higher in non-pregnant cows compared to

pregnant animals ( $331.39 \pm 14.3$  vs.  $288.98 \pm 15.6$   $\mu\text{mol/L}$ ;  $p < 0.05$ ). However, no significant differences in NEFA levels were observed between the two groups at the other sampling time points (one week pre-calving and 10 days

post-calving;  $p > 0.05$ ). These findings suggest that lower NEFA concentrations at one month postpartum may be associated with improved reproductive performance and a higher likelihood of pregnancy at first insemination.

**Table 4.** Comparison of mean NEFA concentrations ( $\mu\text{mol/L}$ ) at different time points around parturition in pregnant and non-pregnant cows

Time relative to parturition	Pregnant (n=9)	Non-pregnant (n=21)	p-value
One week pre-calving	$360.48 \pm 41.67$	$342.15 \pm 24.51$	0.8
10 days post-calving	$676.59 \pm 77.1$	$679.85 \pm 57.6$	0.5
One-month post-calving	$288.98 \pm 15.6$	$331.39 \pm 14.3$	0.03

Values are presented as mean  $\pm$  SEM. A p-value less than 0.05 indicates statistically significant differences between groups.

### Calving to First Insemination Interval

To evaluate the relationship between metabolic status and reproductive performance, cows were categorized based on calving to first insemination interval (CFI) into two groups: early-inseminated (CFI  $\leq 75$  days; n=24) and late-inseminated (CFI  $> 75$  days; n=6).

As presented in Table 5, serum NEFA concentrations at 10 days postpartum were significantly lower in early-

inseminated cows compared to late-inseminated animals ( $651.6 \pm 52.2$  vs.  $915.87 \pm 92.5$   $\mu\text{mol/L}$ ;  $P = 0.02$ ). These findings indicate that elevated NEFA levels at 10 days post-calving are associated with an extended calving to first insemination interval ( $p < 0.05$ ). No significant differences in NEFA concentrations were observed between the two groups at other sampling time points ( $p > 0.05$ ).

**Table 5.** Serum NEFA concentrations ( $\mu\text{mol/L}$ ) in cows with different calving to first insemination intervals

Time relative to parturition	CFI $\leq 75$ days (n=24)	CFI $> 75$ days (n=6)	p-value
One week pre-calving	$382.09 \pm 29.1$	$393.01 \pm 82.04$	0.5
10 days post-calving	$651.6 \pm 52.2$	$915.87 \pm 92.5$	0.02
One-month post-calving	$216.2 \pm 11.3$	$244.15 \pm 34.5$	0.6

Values are presented as mean  $\pm$  SEM. CFI = Calving to first insemination interval. A p-value less than 0.05 indicates statistically significant differences between groups.

### Calving to Conception Interval

Cows were categorized based on calving to conception interval (CCI) into three groups: short (CCI  $< 80$  days; n=13), intermediate (CCI = 80-150 days; n=5), and extended (CCI  $> 150$  days; n=12). As shown in Table 6, the

lowest postpartum NEFA concentrations were observed in cows that conceived before 80 days postpartum. However, statistical analysis revealed no significant differences in NEFA concentrations among the three groups at any sampling time point ( $p > 0.05$ ).

**Table 6.** Serum NEFA concentrations ( $\mu\text{mol/L}$ ) in cows with different calving to conception intervals

Time relative to parturition	CCI $< 80$ days (n=13)	CCI 80-150 days (n=5)	CCI $> 150$ days (n=12)	p-value
One week pre-calving	$375.48 \pm 37.1$	$181.35 \pm 52.0$	$383.6 \pm 52.1$	0.8
10 days post-calving	$633.17 \pm 75.3$	$731.83 \pm 87.7$	$685.2 \pm 67.0$	0.4
One-month post-calving	$201.53 \pm 15.1$	$226.6 \pm 21.6$	$229.86 \pm 18.9$	0.7

Values are presented as mean  $\pm$  SEM. CCI = Calving to conception interval. No significant differences were observed among groups at any time point ( $p > 0.05$ ).

### Number of Inseminations per Conception

Cows were classified according to the number of inseminations required for conception into two groups:  $\leq 3$  inseminations (n=23) and  $> 3$  inseminations (n=7). As presented in Table 7, no statistically significant differences in NEFA concentrations were observed between the two groups at any sampling time point ( $p > 0.05$ ). However, cows that conceived within  $\leq 3$  inseminations consistently

demonstrated lower mean NEFA concentrations across all sampling periods compared to those requiring  $> 3$  inseminations.

## Discussion

The objective of this study was to investigate the dynamics of non-esterified fatty acids (NEFA) during the transition

period and their association with key reproductive performance indicators in Holstein dairy cows. Our findings strongly support the premise that serum NEFA concentrations are not merely a reflection of negative

energy balance (NEB) but also a potent predictor of subsequent reproductive outcomes.

**Table 7.** Serum NEFA concentrations ( $\mu\text{mol/L}$ ) in cows requiring different numbers of inseminations per conception

Time relative to parturition	> 3 inseminations (n=7)	$\leq$ 3 inseminations (n=23)	p-value
One week pre-calving	386.01 $\pm$ 63.2	382.34 $\pm$ 32.45	0.4
10 days post-calving	707.72 $\pm$ 89.6	669.95 $\pm$ 57.81	0.5
One-month post-calving	233.83 $\pm$ 23.2	214.59 $\pm$ 12.69	0.2

Values are presented as mean  $\pm$  SEM. No significant differences were observed between groups at any time point ( $p > 0.05$ ).

### Association between NEFA Dynamics and Energy Balance

The observed pattern of serum NEFA concentration, characterized by a significant increase immediately after parturition and a peak on day 10 postpartum, is a classic metabolic hallmark of the transition period. This pattern is physiologically logical; the onset of lactation creates a massive energy demand, while concurrent reduced dry matter intake (DMI) precipitates a severe NEB. The subsequent mobilization of adipose tissue triglycerides and the release of NEFA into circulation represent a fundamental adaptive response to meet this energy deficit (10, 11). The gradual decline in NEFA concentrations towards one month postpartum, as noted in our study, typically signals a shift toward positive energy balance, driven by recovering feed intake and adapting metabolism. This trajectory is in complete agreement with previous work by Ospina et al. (2010) and Luke et al. (2024), who also identified the first two weeks postpartum as the period of peak NEFA concentrations (7, 9).

### NEFA as a Predictor of Reproductive Performance

A pivotal finding of this study was the significant difference in NEFA levels at one month postpartum between cows that conceived at the first insemination and those that did not. Our findings allow for a predictive interpretation: lower serum NEFA concentrations at one month postpartum in cows that became pregnant at the first insemination act as a positive predictive indicator of successful conception, whereas elevated NEFA reflects a negative predictive value, being associated with a reduced likelihood of pregnancy at first insemination. This reinforces the importance of monitoring NEFA not only as a metabolic marker but also as a practical predictor of reproductive success, allowing early identification of cows at risk of subfertility. It should be noted that NEFA at day 10 postpartum did not show a significant association with fertility, likely because all cows generally exhibit elevated NEFA during early lactation due to the onset of lactation and NEB. By day 30 postpartum, cows that have restored their energy balance tend to show lower NEFA levels, whereas those still in NEB maintain higher NEFA, making day 30 a more reliable predictor of reproductive performance. The relatively small sample size ( $n=30$ ) may also have limited the ability to

detect differences at earlier time points. This scenario, i.e., high NEFA in very early lactation due to NEB, followed by partial metabolic recovery in some cows, is supported by previous reports showing elevated NEFA in early postpartum associated with NEB, and that restoration of energy balance leads to normalization of NEFA (12, 13). This conclusion is strongly reinforced by previous reports (5, 7), highlighting the mechanistic link between elevated NEFA, prolonged NEB, and impaired reproductive function, as further discussed below.

Similarly, Karimi Dehkordi et al. (2016) reported that critical thresholds of NEFA and  $\beta$ -hydroxybutyrate in transition dairy cows can serve as reliable predictors of first-service conception rate (14). Persistently elevated NEFA in non-pregnant cows indicates a prolonged and unfavorable metabolic state (NEB), which critically undermines reproductive efficiency. The detrimental effects are likely multifactorial. High circulating NEFA levels have been demonstrated to directly impair ovarian function by disrupting follicular steroidogenesis and compromising oocyte quality and developmental competence, ultimately leading to reduced embryo quality and lower conception rates (15, 16). Pishvaei et al. (2021) reported that elevated ketone levels negatively affected estrogen and progesterone concentrations, ovulatory follicle size, estrus expression, and overall fertility in Holstein cows (17). Therefore, cows failing to resolve their NEB status in a timely manner are metabolically ill-prepared to support the high-energy processes of a quality estrous cycle and successful establishment of pregnancy. This conclusion is strongly reinforced by the findings of Kia et al. (2023) and Ospina et al. (2010), who reported a clear negative correlation between high postpartum NEFA and pregnancy per artificial insemination (5, 7).

Furthermore, our results establish that NEFA concentration on day 10 postpartum is a robust predictor of the calving-to-first-insemination interval. Cows with higher NEFA at this critical juncture experienced a delayed first insemination ( $>75$  days). This underscores the profound impact of early-lactation metabolic health on the resumption of ovarian cyclicity. Severe NEB is known to delay the first postpartum ovulation and can manifest as silent or sub-estrous cycles, often undetectable to herd managers (2). The mechanistic pathway likely involves the suppressive effect of elevated NEFA on the hypothalamic-

pituitary-ovarian axis, inhibiting the pulsatile secretion of GnRH and, consequently, luteinizing hormone (LH) (2, 4). Since LH pulses are indispensable for final follicular maturation and ovulation, their disruption directly contributes to a delayed resumption of cyclicity. This metabolic stress is often compounded by its association with other periparturient disorders, such as subclinical ketosis, metritis, and retained placenta, which further negatively impact uterine health and the return to estrus (18). Our results are thus consistent with the report by Wang et al. (2022), who linked the intensity of early NEB to a delayed return to estrous cyclicity (6). Consequently, monitoring NEFA around day 10 postpartum can serve as a valuable early-warning tool, allowing for targeted management interventions for at-risk cows before reproductive performance is significantly compromised.

Although the study did not find statistically significant differences in NEFA profiles for groups based on calving-to-conception interval or number of inseminations, a consistent and clinically relevant trend was evident. Across all measurement times, the lowest mean NEFA concentrations were invariably recorded in cows that conceived sooner (<80 days) or required fewer inseminations ( $\leq 3$ ). The lack of statistical significance is likely attributable to the relatively limited sample size ( $n=30$ ), which reduces the statistical power to detect differences for these more extended endpoints. Nevertheless, this persistent trend powerfully reinforces the overarching hypothesis that a superior metabolic status—characterized by lower NEFA concentrations throughout the transition period—is a critical determinant of higher overall reproductive efficiency. This concept of using serial metabolic evaluations to build a more complete picture of a cow's reproductive potential is supported by Luke et al. (2024) (9).

The primary limitation of this study is its sample size ( $n=30$ ), which may have constrained the statistical power to detect significant differences for all reproductive parameters, particularly the calving-to-conception interval and number of inseminations per pregnancy. Future studies with larger cohorts are warranted to confirm these trends and further elucidate the relationship between prolonged NEB and broader fertility metrics.

The practical implication of our findings is substantial. Monitoring NEFA concentrations, particularly at the critical 10-day postpartum mark, provides a powerful and objective tool for predicting herd reproductive performance and identifying cows at risk of subfertility. High NEFA at this time is an unequivocal alert of severe NEB, which is highly likely to translate into delayed first insemination and reduced conception rates.

Implementing this monitoring in commercial herds enables targeted interventions, such as strategic dietary adjustments, the use of glucogenic or lipogenic feed additives, and enhanced health monitoring for high-risk animals. The key to simultaneous improvements in production and fertility lies in minimizing the depth and duration of NEB through optimal nutritional management, including improving forage quality, utilizing bypass fats,

and ensuring a smooth transition from the dry period to lactation. Moreover, based on these metabolic indicators, a strategy of deliberately delaying the first insemination for cows with excessively high NEFA until their metabolic status improves and regular cyclicity is established could prove economically beneficial by improving conception rates and reducing the number of inseminations required.

## Conclusion

Overall, the findings of this study indicate that monitoring changes in NEFA levels during the transition period provides valuable insight into the metabolic status of Holstein dairy cows and their relationship with reproductive performance. Evaluating NEFA dynamically, rather than relying on a single measurement, offers a more accurate understanding of a cow's ability to adapt to the metabolic demands of early lactation. Cows that failed to resolve their negative energy balance in a timely manner showed a lower likelihood of successful conception. These results highlight the importance of using metabolic profiling as a supportive tool in management decisions to improve herd productivity and animal health. Future studies with larger sample sizes and additional metabolic indicators may further refine predictive thresholds and strengthen these conclusions.

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## Authors' Contributions

**Maryam Karimi-Dehkordi:** Study design, supervision of experiments, data analysis, manuscript writing and final revision; **Forogh Mohammadi:** Conducting experiments, data collection, drafting the initial manuscript; **Hosein Soltani:** data interpretation and technical support; **Shahin Nejat Dehkordi:** Scientific consultation and manuscript review.

## Data Availability

The datasets generated and/or analyzed during the current study are available from the corresponding author on reasonable request.

## Ethical Approval

All animal procedures were performed in accordance with the ethical guidelines of Islamic Azad University of Shahrekord branch.

## Conflict of Interest

The authors declare that they have no competing interests.

## Consent for Publication

Not applicable

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