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Assessment of Some Biochemical and Reproductive Parameters in Cyclic and Acyclic Holstein Cows at the End of Voluntary Waiting Period

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This study aimed to determine certain biochemical and reproductive parameters in cyclic and acyclic Holstein cows at the end of the voluntary waiting period. A total of 30 Holstein cows, were used in the study. Among these animals, those with a corpus luteum (CL) on their ovaries in either of the consecutive examinations performed on days 45 and 55 postpartum, and had a blood progesterone level >1 ng/mL, were considered cyclic (n=15); Those with no CL on their ovaries in either examination and with a blood progesterone level <1 ng/mL were considered acyclic (n=15). On day 55 postpartum, blood samples were obtained from each cow. Parity and body condition score (BCS) were recorded for each animal. The serum samples were collected and stored at –80°C until analysis. β-hydroxybutyrate (BHB), non-esterified fatty acids (NEFA), glucose, cholesterol, triglyceride and progesterone levels were measured. The first estrus after the voluntary waiting period was determined based on increased locomotor activity, uterine tone, and the presence of a Graafian follicle on the ovary. Farm records were used to evaluate the reproductive parameters. It was found that the intervals from calving to first estrus, calving to first insemination, and calving to conception were significantly shorter in cyclic cows compared to acyclic cows. When comparing biochemical parameters, parity, and BCS, no significant differences were found between the groups. In conclusion, while there were no significant differences in biochemical parameters, parity, or BCS between cyclic and acyclic cows, reproductive parameters were significantly improved in cyclic cows.

Key Words: *Biochemical, reproductive, cyclicity, postpartum, cow*

Siklik ve Asiklik Holştayn İneklerde Gönüllü Bekleme Süresi Sonunda Bazı Biyokimyasal ve Üreme Parametrelerinin Değerlendirilmesi

Bu çalışmada, gönüllü bekleme periyodu sonunda siklik ve asiklik Holstein ineklerde belirli biyokimyasal ve üreme parametrelerinin belirlenmesi amaçlandı. Çalışmada toplam 30 Holstein inek kullanıldı. Bu hayvanlar, doğum sonrası 45. ve 55. günlerde yapılan ardışık muayenelerden birinde ovaryumlarında korpus luteum (CL) bulunan ve kan progesteron seviyesi >1 ng/mL olan inekler siklik (n= 15), her iki muayenede ovaryumlarında CL olmayan ve kan progesteron seviyesi <1 ng/mL olan inekler asiklik (n=15) olarak kabul edildi. Doğum sonrası 55. günde her inekten kan örnekleri alındı. Her hayvan için parite ve vücut kondisyon skoru (VKS) kaydedildi. Serum örnekleri toplanarak ve analiz edilene kadar -80°C'de saklandı. β-hidroksibutirat (BHB), esterlenmemiş yağ asitleri (NEFA), glukoz, kolesterol, trigliserid ve progesteron düzeyleri ölçüldü. Artan lokomotor aktivite, artan uterus tonusu ve ovaryumda Graaf folikülünün varlığına dayanarak, gönüllü bekleme süresinden sonraki ilk östrus belirlendi. Üreme parametrelerini belirlemek için çiftlik kayıtları kullanıldı. Siklik ineklerde buzağılamadan ilk östrusa, buzağılamadan ilk tohumlamaya ve buzağılamadan gebe kalmaya kadar geçen sürelerin asiklik ineklere kıyasla anlamlı derecede daha kısa olduğu bulundu. Diğer biyokimyasal parametreler, parite ve BCS karşılaştırıldığında, gruplar arasında anlamlı bir fark bulunmadı. Sonuç olarak, siklik ve asiklik inekler arasında biyokimyasal parametreler, parite veya BCS'de anlamlı bir fark bulunmazken, üreme parametreleri siklik ineklerde daha iyiydi.

Anahtar Kelimeler: *Biyokimyasal, reprodüktif, siklik, pospartum, inek*

Introduction

Reproductive efficiency is one of the most critical factors that influences profitability in dairy cow farming. The calving interval is considered one of the most important parameters used to assess reproductive performance in cows. For dairy cattle, the economically optimal calving interval is generally accepted to be one year (1, 2). For reaching this economically optimal 365-day calving interval, it is crucial that ovarian cyclicity returns early in the postpartum period (pp), an accurate estrus can be detected, and conception rates at first insemination are high (3).

The period between calving and first insemination is referred to as Voluntary Waiting Period (VWP) (4, 5). Maintaining a 12-month calving interval in dairy cows requires the animal to conceive again as soon as possible after calving. To reach such a calving interval, uterine involution and re-establishment of endocrine function must be completed during the VWP (6,7). The VWP should be preferably around 45-50 days (8, 9). In Holstein cows, the first wave of follicular growth begins around two weeks after calving about In about 30% to 40% of these cows, ovulation occurs ovulation occurs

between days 16 and 20 pp in about 30% to 40% of these cows. However, 30% to 40% of cows ovulate between 30 and 50 days pp, while about 20% to 40% remain anovulatory until 50 to 60 days pp (10-12).

An early return of ovarian cyclicity improves reproductive efficiency, leading to greater milk yield (13). In contrast, delayed ovarian cyclicity during the pp period negatively affects reproductive performance (14). The onset of ovarian cyclicity, the first ovulation, and the continuation of regular estrous and ovulatory cycles after calving are influenced by several factors, including breed, parity, season, body condition score (BCS), postpartum diseases, and nutrition (15).

Although extended VWP is known to impair fertility, there isn't enough research on this topic in Holstein cows raised under Turkish conditions. To adress this gap, this study aimed to evaluate certain biochemical parameters that may influence the length of the VWP in Holstein cows and to investigate the effects of an extended VWP on some reproductive parameters.

Materials and Methods

Research and Publication Ethics: Permission for the study was received from Tekirdağ Namık Kemal University Animal Experiments Local Ethics Committee (dated 06.03.2025) and numbered T2025-2.

Animals, Nutrition and Management Conditions: The study included 30 healthy Holstein cows aged 2-6 years from a private dairy farm in Tekirdağ Province, Türkiye. All the cows had recently given birth and had no history of puerperal illnesses such as dystocia, retained placenta, endometritis, laminitis, or displaced abomasum. All animals received the same care, food, and reproductive management, and were fed ad libitum twice a day with total mixed rations designed for the transition period. Clean drinking water was continuously available to the animals. All cows included in the study were with normal BCS values.

Study Design: All cows in the study were examined twice, on postpartum (pp) days 45 and 55, by rectal and ultrasonographic examination (Hasvet WED-3100V) at a 10-day intervals. Cows that had a corpus luteum (CL) detected on at least one of these examinations and a blood progesterone level >1 ng/mL were classified as cyclic (n= 15), while cows with no CL detected in either examination and a progesterone level <1 ng/mL were considered acyclic (n= 15). On the day 55 pp, which is the day of the second examination, blood samples were collected from the tail vein of all animals. The age and BCS of each animal were recorded. The blood samples were centrifuged at 3000 rpm for 15 minutes, and the serum was separated and stored at –80°C until analysis. β-hydroxybutyrate (BHB), non-esterified fatty acids (NEFA), glucose, cholesterol, and triglyceride levels were measured using an autoanalyzer (Randox, RX Imola, Crumlin, United Kingdom). Progesterone was determined using the chemiluminescence technique with an autoanalyzer (Roche Cobas e 701). The first estrus following the VWP was determined based on increased activity measured by pedometer, increased uterine tone on rectal palpation, and the presence of a Graafian follicle in the ovaries. Reproductive performance of the animals were evaluated using the farm records to determine the number of days from calving to first insemination and from calving to conception for each animal included in the study.

Statistical Analysis: Normality of the data was assessed using the Shapiro-Wilk test. Parameters showing normal distribution were analyzed using the parametric unpaired t-test, while those not normally distributed were analyzed with the non-parametric Mann-Whitney test. All analyses were conducted using the GraphPad Prism 9.4.1 software. Statistical significance was set at $p<0.05$.

Results

There were no statistically significant differences between the groups in terms of biochemical parameters (BHB, NEFA, glucose, cholesterol, triglycerides) ($p>0.05$) (Table 1). Similarly, parity and BCS showed no statistically significant differences between the groups ($p>0.05$) (Table 1).

In acyclic cows, the intervals from calving to first estrus, calving to first insemination, and calving to conception were found to be significantly shorter compared to cyclic cows ($p<0.01$) (Table 2).

Table 1. Distribution of histopathological parameters in the groups

Parameter	Group	Mean ± SEM	Median (IQR)	*p*-value
BCS	Acyclic	3.52 ± 0.13	3.75 (3.5-4.0)	0.3321
	Cyclic	3.68 ± 0.09	3.5 (3.5-4.0)	
Parity	Acyclic	2.64 ± 0.45	2.0 (1.0-4.0)	0.8663
	Cyclic	2.55 ± 0.28	3.0 (2.0-3.0)	
NEFA (mmol/L)	Acyclic	0.42 ± 0.07	0.37 (0.24-0.56)	0.5774
	Cyclic	0.37 ± 0.05	0.37 (0.23-0.46)	
BHB (mmol/L)	Acyclic	0.76 ± 0.20	0.49 (0.33-0.77)	0.3913
	Cyclic	0.81 ± 0.22	0.59 (0.46-0.76)	
Glucose (mg/dL)	Acyclic	56.76 ± 1.71	58.71 (50.96-62.41)	>0.9999
	Cyclic	56.86 ± 1.33	57.58 (52.68-59.86)	
Cholesterol (mg/dL)	Acyclic	96.24 ± 15.02	92.68 (61.29-115.7)	0.2372
	Cyclic	127.00 ± 20.34	92.76 (83.03-166.3)	
Triglyceride (mg/dL)	Acyclic	8.83 ± 1.24	8.54 (5.10-12.45)	0.2703
	Cyclic	10.56 ± 1.38	10.08 (7.21-14.17)	

BCS: Body Condition Score, **NEFA:** Non-esterified fatty acids, **BHB:** Beta-hydroxybutyrate.

Table 2. Comparison of reproductive parameters between the groups

Parameter	Group	Mean ± SEM	Median (IQR)	*p*-value
Calving to First Estrus (days)	Acyclic	170.20 ± 18.97	150 (120-213)	<0.0001
	Cyclic	63.09 ± 5.30	60 (48-80)	
Calving to First Insemination (days)	Acyclic	172.70 ± 17.11	150 (140-200)	<0.0001
	Cyclic	72.55 ± 4.35	70 (60-85)	
Calving to Conception (days)	Acyclic	187.70 ± 15.83	165 (150-230)	<0.0001
	Cyclic	87.36 ± 4.81	90 (80-100)	

Discussion

The postpartum period is critical for the reproductive performance and overall health of dairy cows. The complex interaction between hormonal dynamics and metabolic status plays a significant role in determining reproductive outcomes. The aim of this study was to explore the effects of selected biochemical and reproductive parameters at the end of the voluntary waiting period on reproductive efficiency in cyclic and acyclic cows.

Changes in BCS during the transition period are regarded as markers of negative energy balance (NEB) (16). Cows that resume cyclic activity early after calving have been reported to have higher BCS compared to acyclic cows. The reason for this is that cows with a higher BCS usually have better nutritional condition and sufficient gonadotropin secretion (17, 18). However, the present study found no significant difference in BCS between cyclic and acyclic cows (p>0.05). The similarity in nutrition and management strategies between both groups could explain why this difference did not reach statistical significance.

It has been reported that parity has a significant effect on the resumption of ovarian activity after calving. Cows that gave birth for the first time typically experience a later onset of ovarian cyclicity compared to those that calved multiple times (19-21). However, in the present study, no significant difference was found between the parities of cyclic and acyclic cows (p>0.05). The reason for this could be the fact that the animals in the study were not grouped as primiparous and multiparous and were selected with similar parities.

Non-esterified fatty acids and BHB are considered key indicators of NEB. It has been reported that there is a relationship between the resumption of ovarian cyclicity and NEB after calving (22). Elevated levels of NEFA and BHB, which are indicators of NEB, have been shown to negatively affect ovarian activity (23, 24). However, other studies have also reported that NEFA and BHB do not directly affect the resumption of ovarian cyclic activity (25). In the present study, no significant difference was found between the NEFA and BHB levels in cyclic and acyclic cows (p>0.05). These results are consistent with the findings of Teixeira et al. (25). The lack of disparities between the groups suggests that both were controlled with efficient feeding regimens. Differences between studies could be attributed to the number of animals in each group and the feeding regimens they followed.

The effects of blood glucose levels on postpartum ovarian cyclic activity are not exactly clear, and there are some inconsistencies in the studies on this topic (13, 26,

27). Some studies found no link between serum glucose levels and the delay in postpartum ovarian cyclic activity (13, 26, 27). Others (28, 29), however, argue that glucose is essential for normal ovarian dynamics in dairy cows, with cyclic cows showing much higher glucose levels than acyclic cows. The current study found no significant difference in glucose levels between the groups (p>0.05). These findings agree with those of Jeong et al. (13), Garverick et al. (26), and Obese et al. (27). The absence of changes in glucose concentrations is likely due to the strict homeostatic control over glucose levels (30).

Cholesterol is an important substrate for the synthesis of ovarian sex hormones and has a significant effect on follicular development. It has been reported that an imbalance in cholesterol homeostasis may have a negative impact on ovarian structure and function (31). In the present study, although no significant difference was found in cholesterol and triglyceride levels (p>0.05), higher cholesterol levels were seen in cyclic cows. Cholesterol also plays a critical role in the synthesis of steroid hormones, which could indicate that hormonal activity is more intense in cyclic cows. This increase in cholesterol could be related to the support of follicular development and luteal function. It may also reflect the positive effects of effective nutritional interventions on lipid metabolism. Higher cholesterol levels may suggest higher hormonal activity, which is beneficial to reproductive health. Cholesterol is a precursor to the synthesis of steroid hormones such as progesterone and estrogen; therefore, higher levels could suggest increased hormonal activity (32). Subtle differences in our findings suggest that lipid metabolism was regulated by successful nutritional interventions.

The resumption of ovarian cyclicity after calving is important for protecting reproductive health in dairy cows. It affects calving intervals and reproductive performance directly, which in turn affects the profitability of dairy farms (15, 33). Delays in postpartum ovarian cyclic activity also extend the VWP, and cows with a longer VWP have lower calving frequency (34). Cows with regular postpartum ovarian cyclic activity are found to have better reproductive performance than cows with delayed postpartum ovarian activity (14). Shrestha et al. (14) reported that the calving to first insemination interval was 66.7 days in cyclic cows, compared to 92.7 days in anestrous cows. In the current study, the calving to first insemination interval was 72.55 days in cyclic cows and 172.7 days in acyclic cows. Both the first insemination and conception intervals were significantly earlier in cyclic cows. On the other hand, these intervals were delayed in acyclic cows. For cyclic cows, the calving to conception period was 87.36 days, and for acyclic cows, it was 187.70 days. Similar to our study, Ledoux et al. (35) and Gautam et al. (36) found that cows with delayed ovarian activity had longer calving to first insemination and calving to conception intervals than cows with normal ovarian function.

In conclusion, in the current study, no significant differences were found between cyclic and acyclic cows in terms of biochemical parameters, parity, or BCS. However, cyclic cows had significantly higher calving to first estrus, calving to first insemination, and calving to conception intervals compared to acyclic cows. These findings highlight the importance of monitoring cyclicity in herd management, further demonstrating that cows that return to cyclical activity at the end of the VWP have better reproductive performance.

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