Journal homepage
www.ajas.uoanbar.edu.iq
Anbar Journal of Agricultural Sciences
(University of Anbar – College of Agriculture)

ESTRUS BEHAVIOR CHANGES DURING THE FIRST, SECOND, AND ≥3RD POSTPARTUM OF LACTATING DAIRY COWS

H. M. H. Zebari [1,2]* **M. S. Rutter [2]** **E. Bleach [2]**

[1] **Department of Animal Production, College of Agricultural Engineering Sciences, University of Duhok, Kurdistan, Iraq.**
[2] **Department of Agriculture and Environment, Harper Adams University, Newport, Shropshire, UK.**

***Correspondence to:** Hawar M. H. Zebari, Department of Animal Production, College of Agricultural Engineering Sciences, University of Duhok, Kurdistan, Iraq.
Email: hawar.mikahil@uod.ac

Article info

Received: 2025-03-08
Accepted: 2025-04-25
Published: 2025-06-30

DOI-Crossref:
10.32649/ajas.2025.187603

Cite as:
Zebari, H. M. H., Rutter, M. S., and Bleach, E. (2025). Estrus behavior changes during the first, second, and ≥3rd postpartum of lactating dairy cows. Anbar Journal of Agricultural Sciences, 23(1): 695-710.

Abstract

This study evaluated the changes in estrus behavior during the first, second, and third postpartum (PP) periods of dairy cows. Milk samples were collected from thirty lactating Holstein Friesian cows on Mondays, Wednesdays, and Fridays to determine concentrations of the progesterone (P4) hormone in them. IceQubes were used to measure the animals' activity 3 days before, on the day of estrus and 3 days after estrus. A roughage intake control (RIC) system recorded feeding behavior. The cows were identified to be estrus when the P4 concentrations in their milk were above 3ng/ml 2 to 3 days before and 5 days after estrus. On the day of estrus, more steps were taken by the animals (P<0.001) but lying time and bouts, dry matter intake (DMI), and feeding behavior were lower (P<0.001) compared to other days. Butting of other cows (P=0.010), sniffing udders (P<0.001), and total agonistic behaviors (P=0.016) were lower in the 1st and 2nd estrus compared to the ≥3rd estrus PP. In conclusion, the number of steps taken by the cows increased, while lying time, lying bouts, and feeding behavior declined on the day of estrus. Butting, sniffing udders, and total agonistic interaction during ≥3rd estrus occurred more than during the 1st or 2nd PP estrus. Activity monitoring for estrus detection is recommended for use in dairy cattle farms.

Keywords: Lactating cows, Steps, activity, Estrus postpartum, Feeding behavior.

تغيرات سلوك الشبق خلال الشبق الأولى والثانية والثالثة او اكثر بعد الولادة لدى الأبقار الحلوب المرضعة

هوار ميكائيل حسن الزيبارى [1,2]* ماركـ ستيڤن روتر [2] ئيما بليچ [2]

[1] قسم الانتاج الحيواني، كلية علوم الهندسة الزراعية، جامعة دهوك.

[2] قسم الزراعة والبيئة، جامعة هاربر ادامس، المملكة المتحدة.

***المراسلة الى:** هوار ميكائيل حسن الزيبارى، قسم الانتاج الحيواني، كلية علوم الهندسة الزراعية، جامعة دهوك، العراق.

البريد الالكتروني: hawar.mikahil@uod.ac

الخلاصة

استهدفت هذه الدراسة إلى تقييم تغيرات سلوك الشبق خلالِ شبق الأولى والثانية والثالثة او اكثر بعد الولادة لدى الأبقار الحلوب المرضعة. استُخدمت ثلاثون بقرة هولشتاين فريزيان مرضعة في هذه التجربة. جُمعت عينات من الحليب لمدة ثلاثة مرات في الاسبوع، تحديداً أيام الاثنين والأربعاء والجمعة لتحديد تركيز هرمون البروجسترون (P4) في الحليب. استُخدمت أجهزة IceQubes لقياس نشاط الأبقار قبل ثلاثة أيام من الشبق، ويوم الشبق، وبعد ثلاثة أيام من الشبق. استُخدم نظام التحكم في تناول العلف الخشن (RIC) لتسجيل سلوك التغذية (مدة التغذية اليومية وعدد مرات زيارة الأبقار للرضاعة). تم تحديد حالة البقرة في حالة شبق عندما تجاوز تركيز هرمون البروجسترون (P4) في الحليب 3 نانوغرام/مل لمدة يومين إلى ثلاثة أيام قبل الشبق وخمسة أيام بعده. حيث كانت يوم الشبق، الخطوات أعلى ($P < 0.001$) مقارنةً بالأيام الأخرى. ومع ذلك، في يوم الشبق، كانت مدة الاستلقاء، ومرات الاستلقاء، وكمية المادة الجافة المستهلكة (DMI) وسلوك التغذية أقل ($P < 0.001$) مقارنةً بالأيام الأخرى. كان نطح الأبقار الأخرى ($P = 0.010$)، وشم الضرع ($P < 0.001$)، السلوكيات العدوانية ($P = 0.016$) أقل في الشبق الأول والثاني مقارنةً بالشبق الثالث أو أكثر. وفي النهاية، ازداد عدد خطوات المشي التي اتخذتها الأبقار، بينما انخفض وقت الاستلقاء، ومرات الاستلقاء، وسلوك التغذية في يوم الشبق. كان نطح الأبقار الأخرى، وشم الضرع، السلوكيات العدوانية خلال الشبق الثالث أو أكثر أعلى منه في الشبق الأول أو الثاني. يوصي الباحث باستخدام مراقبة النشاط للكشف عن الشبق في مزارع الأبقار الحلوب.

كلمات مفتاحية: الأبقار الحلوبة، الخطوات، النشاط، الشبق بعد الولادة، سلوك التغذية.

Introduction

Fertility rates among dairy cows have been declining over the last few decades (37). In the UK dairy herd, the decline is recorded to be 1% per year (31) while for European dairy cattle it is due to higher milk yields/cow (38) with genetic selection focusing more on increasing milk yield rather than for fertility (22). Estrus detection plays an important role in optimizing fertility in dairy cows (29).

Globally, most dairy farmers use artificial insemination (AI) to get more pregnant cows (7). At ovulation, fertilizing the oocyte by sperm depends on delivery of semen into the reproductive tract of the cows through successful AI and to produce a feasible embryo which is guaranteed to gestate (6). The exact detection of estrus determines the optimal AI time (23). Thus, the accurate recognition of estrus is a key factor determining reproductive efficiency in dairy cows and improving pregnancy rates (37 and 40).

Recently, lactating dairy cows show standing estrus considering absolute estrus signs have declined from 74% to 44.9% as a result of increasing milk yield (8 and 11). Thus, the detection of estrus is a major issue facing lactating dairy cows despite the increased understanding and knowledge of their reproductive physiology and the use of automated estrous detection (AOD) services (29 and 41). In cases where exact estrus detection is achieved, calving intervals in dairy cows can be reduced from 389 to 365 days (15) while conception rates can be maximized (29 and 34).

The average length of the estrous cycle in dairy cows is 21 days (ranging 18 to 24 days) and estrous behavior durations vary from 6 to 33 hours with an average of 8 hours for the lactating cow (12). Various factors contribute to the length and variations in estrous expression in dairy cattle, which results in poor estrous detection (19). These include management factors (8), social interaction (16), environmental factors (33), milk yield (9), age and parity (38), nutrition and BCS (17), days in milk and number of estrus PP (40).

During behavioral estrus, ovarian follicles produce estrogens that influence the behavioral centers in the brain of the animal (11). This gives rise to the primary signs of estrous such as standing to be mounted (10), which lead to the secondary signs such as anogenital sniffing, head and side mounting, restlessness, bellowing, chin resting, and mounting attempts (14). This leads to further secondary signs such as lying time (18), and steps taken (8), changes in dry matter intake (DMI), and the duration of feeding. Thus secondary signs can be used as indicators of dairy cow’s estrous expression (27).

Estrus activity monitoring systems can only identify about 70% of estrus in Holstein Friesian dairy cows (13) while 20% are considered anovular and 10% ovulate, not displaying any estrous behavioral signs (13). Dolecheck (8 and 9) detected only about 62.1% of accurate estrus by visual observation (VO) while 65.5% of estrus was detected through automated activity monitoring (AAM) technology of Holstein cows. A rise in the rate of silent estrus in early lactation cows is attributed to the increase in milk yield from 28 to 36 kg/d (40). Dairy cows which lose more body weight during lactation and less body condition scores (BCS) during the early PP period take 30 days longer to express the 1st behavioral estrus PP (4). This means that despite the use of estrus detection aids such as activity monitors, estrus detection in lactating dairy cattle is difficult. Therefore, this study aimed to evaluate whether the primary and secondary signs of estrus behavior in lactating Holstein Frisian cows can be affected by 1st, 2nd, and 3rd PP estrus.

Materials and Methods

The study was conducted between 6 June and 9 August 2016 at Harper Adams University dairy unit farm, Newport, Shropshire, TF10, 8NB, UK.

Animal management: Holstein-Friesian cows (n=30) during lactating period with days in milk (DIM) of 29±6.3 days were used in this experiment. Their average parity, body weight and milk yield were 2.5±1.1, 637.0±60.0 kg and 35.8±1.8 kg/d, respectively. At the beginning of the experiment, their body condition scores (BCS) were 2.9±0.28 according to AHDB Dairy (BCS scale 1-5) 2014 while their locomotion score was 2.0±0.58 (Scale 1-5) (5). The animals were housed in a free stall barn (34 cubicles of 2.7 x 1.2m) covered with thick rubber mattresses (3 cm) and 2 passageways with grooved concrete; 6 x 50m width around 10.8m2/cow. Sawdust was used for covering the cubicles every two days. An automatic scraper was used to clean the concrete passageways every 4-5 hours daily. The cows were milked twice a day at 05:00 and 16:30 using a 40-point internal milking rotary parlour (Westfalia, GEA Milking System, Germany). About 30-40 minutes were allocated for milking.

Diet composition and feed intake: Thirty bins of the roughage intake control system (RIC) were used to feed the cows and an automated feed recording system (1.0x0.9x0.8m; Insentec B.V. Marknesse, the Netherlands) noted feed intake. Data were collected from 06 June to 19 August 2016. The first week of the study was considered the adaptation period to ensure that each studied cow can feed successfully without support through RIC bins. About 65kg fresh weight of total mixed ration (TMR) was supplied at 08:30 am daily, which were sufficient for ad libitum eating. Refused feed was removed three times per week on Monday, Wednesday, and Friday at 08:00 am and the RIC bins cleaned before fresh feed was allocated. Three water troughs were used to provide water ad libitum. At the time of feeding, the TMR samples were collected daily from the bins then oven-dried overnight at 105 °C immediately after collection of feed samples to constant weight according to AOAC (2012;899.02) for measuring DM. The nutrient content of the TMR was supplied according to Zebari et al. (40).

Data Collection:

Video recording of the behavioral signs of estrus: Video cameras (n=4; Voltek, KTandC Co Ltd, Seoul, SK) were used to monitor and record behavioral signs of spontaneous estrus among the cows. The cameras were fixed about 5.25m directly above the experimental cubicles and passageways to provide a clear view of the experimental animals. A video recorder external hard drive was connected to the cameras. For identification, numbers 1 to 30 were visibly painted on both sides of the cows’ backs. In addition, a clear combination of highlighted tape was used on each studied cow (19). To accurately identify the time and intensity of estrus expression of each cow, video recordings were reviewed in retrospect by researchers. Estrus signs scores were assigned and recorded whenever signs of behavioral estrus were detected by video recording (35), and estrus intensity was determined in accordance with the total number of points scored on the day of estrus.

Activity of cows and feed intake: To monitor the cows' estrus activity, IceQubes (3-axis accelerometer; Ice-Robotics Ltd., Edin., United Kingdom) were attached to their left rear legs (8). The IceQubes monitor and summarize activity in 15-min blocks and generate daily data (number of steps taken, lying time and lying bouts) on the animals. The RIC system recorded daily TMR intake, feeding duration, and number of feed-visits per cow. The DMI was calculated as fresh weight intake of TMR kg/d x TMR dry matter percentage.

Assay of milk progesterone (P4): Whole milk P4 concentrations were measured to identify estrus periods. Approximately 40 ml of milk per cow was collected 3 times a week in the afternoon. Immediately after collection, a single protective tablet (Broad Spectrum Microtabs II, Inc. USA; containing 8mg Bronopol and 0.30mg Natamycin) was added to each milk sample. The preservative tablets were dissolved by inverting the milk sample pots. The milk samples were stored at 4 ºC until the weekly progesterone assay was carried out. They were transported at room temperature and mixed well and then analyzed for P4 concentration using the enzyme immunoassay protocol (Ridgeway Science Ltd., Alvington, Gloucestershire, United Kingdom). Cows were identified to be estrus when the P4 concentrations in the milk exceeded 3ng/ml for 2 to 3 days before estrus and above 5ng/ml for a minimum five days after estrus (2 and 17).

Definition of estrus behavior: A behavioral or silent estrus was identified and classified according to the P4 profile. The cows were considered to be in behavioral estrus when the sum of the reviewed and observed score points from the video camera exceeded 100 points (35).

Definition of number of estrus PP: Each estrus period identified by the progesterone assay of milk and which generated an alert from the accelerometers was classified as the 1st, 2nd, and 3rd estrus PP. Cows in their third and fourth PP estrus were classified into the ≥3rd PP estrus period. Therefore, the number of PP estruses were grouped as first, second, and third or more. The obtained data were statistically analyzed to determine whether the number of estrus post-partum influenced estrus expression activity (behavior) in the lactating cows.

Data set construction: Sixty-one estrus events were identified during the study period and the data from all of them was analyzed. Parameters analyzed included the number of steps/d, lying time (h/d), lying bouts number/day, DMI (kg/d), feeding duration (h/d), and number of daily feed visits. Other estrus signs such as mounting but not standing, flehmen, mucous discharge from vulva mounting or mount attempt, restlessness, and standing to be mounted were also analyzed. Before analysis, Microsoft Excel was used to summarize all parameter data per day into one value. The data for three days before (-3, -2 and -1 or 3DB) were compared with day zero (day of estrus) and three days after (+1, +2 and +3 or 3DA) estrus. Based on the TMR's DM content which was about 39.5%, fresh TMR (kg/d) intakes were transformed into DMI (kg DM/d).

Statistical analyses: Statistical analyses were carried out using GenStat software package (V 18th.18.1.14912 provided by VSN International Ltd, United Kingdom). To compare behavioral and silent estrus, repeated measures (ANOVA) were used. The same analyses were also used to compare between 3DB, days of estrus (0), and 3DA estrus and between the groups x day interaction. Factorial one-way analyses were used to compare between the frequency of sexual, social, and agonistic between first, second and third PP estrus. When a significant interaction was found, a t-test was used to compare the following: lying time, number of step taken each day, daily number of lying bouts, dry matter intake, feeding duration, feed visits from 3DB estrus, day of estrus (0), and 3DA estrus. The comparison between the three PP estruses were analyzed by Tukey test. In addition, Chi-Square tests were used to compare flehmen, mounting or attempt to mount, mucous discharge from vulva, restlessness, mounting but not standing, and standing to be mounted between the PP estruses. Differences between studied parameters were considered as significant at $P<0.05$.

Results and Discussion

Estrus detection in the various PP estrus periods: The estrus proportions detected from the P4 concentration in milk during the first, second and third or more PP estrus periods from the 61 events were 47.5%, 36% and 16.3%, respectively. The proportion of behavioral estrus detected by observation during the three periods was 55.3%, 72.6% and 80% respectively. The results of this experiment agree with Ranasinghe et al. (26) who reported 55.2 *vs* 44.8 %, 76.2 *vs* 23.8% and 78.7 *vs* 21.3 % of behavioral and silent estrus at 1st, 2nd and ≥3rd estrus PP in a herd of lactating Holstein Friesian dairy cows using concentrations of progesterone hormone profile in milk. However, another study on 32 Holstein Friesian cows in Japan by Isobe et al. (17) using the same bases noted that silent estrus occurrences were 83 at 1st, 46 at 2nd and 13% at ≥3rd PP estrus.

Furthermore, a study based on continuously recording mounting activity by radio-telemetry also noted the prevalence of silent estrus being significantly higher at 1st PP estrus (42.1%) than the 2nd at 12.5% (33). The proportion of recognition of behavioral estrus using pedometers were 57, 91 and 95% at first, second and third or more estrus post-partum, respectively, but that from visual observation were 19, 37, and 79%, respectively (11). In lactating dairy cows, the occurrence of silent estrus at 1st estrus PP was higher than for the others depending on the refraction of the concentration of estrogen (26). This may be due to the high concentration of the estrogen hormone circulating in the blood during the late pregnancy stage that results in the refractoriness before the 1st follicular ovulation to estrogen (1).

Other studies suggest that lower expression at 1st PP estrus may also be caused by lower frequency of LH pulses as a result of negative energy balance in early lactation (22). This results in lower estradiol synthesis (4) by the preovulatory follicle and decreases the sensitivity of the hypothalamus to estradiol which leads to a high incidence of silent estrus. However, with progress in PP estrus the proportion of behavioral estrus detection among the cows increased. This is due to several physiological related factors such as hormonal recovery, resumption of ovarian cyclicity, uterine involution completion, and increased estrogen sensitivity of the receptors in the brain of dairy cows (17), in addition to energy balance and nutritional recovery (9).

Activity during different PP estrus periods: On the day of estrus at first PP estrus, the number of steps taken were higher ($P<0.001$; 1502±192 steps) compared to 3DB and 3DA estrus. They were also more (1730±285; $P\leq 0.01$) on day 0 at second PP estrus than other days. The number of steps was also significantly more ($P<0.001$) at 2285±480 on the day of estrus in comparison to 3 days prior-to and after estrus. However, there was no significant different ($P=0.07$) in comparison to day two after estrus (1234±273 steps). There was also no difference ($P=0.233$) among 1st, 2nd and ≥3rd estrus with regard to steps taken on the day of estrus (Figure 1).

Similarly, a previous study found that estrus activity among lactating cows increased with the number of days especially after day 50 post-partum (21). Likewise, Yániz et al. (39) noted increased walking activity during estrus with advancing days post-partum among Holstein-Friesian cows in Spain. A decline in steps taken by cows at 1st estrus post-partum may be due to the low production of estrogen in the developing follicle because of negative energy balance during first stage of high-yielding lactation dairy cows (17).

The cows spent less lying time ($P<0.001$) on the day of estrus at 1st (8.11±0.5h/d), 2nd (7.77±0.5h/d) and ≥3rd (7.25±0.9h/d) PP estrus compared to other days, while there was no influence ($P=0.60$) on the day of estrus. There was also no interaction ($P=0.440$) between the number of PP estrus and time cows spent lying on the day of estrus (Figure 2).

There was also a significant ($P<0.001$) effect of estrus on lying bouts with fewer numbers observed on the day of estrus at first (11.6±1.1 bouts), second (9.55±0.6) and third or more (8.6±1.5) PP estrus than 3DB and 3DA. However, there was no significant ($P = 0.105$) difference in the number of lying bouts on the day of estrus between first, second, and third estrus PP. Numerically, more lying bouts ($P=0.479$) were recorded at first PP estrus compared to the second or third or more periods (Figure 3). On the day of estrus of third and more PP estrus, the animals spent less lying time and had fewer lying bouts compared to the first and second estrus PP. This may be attributed to an increase in walking activity and restlessness (21) on the day of estrus. This experiment also noted a significantly negative correlation ($P < 0.001$) between steps taken and lying time ($y = 0.0.001\ x +9.78$; $r^2 = 0.37$) across the day of estrus.

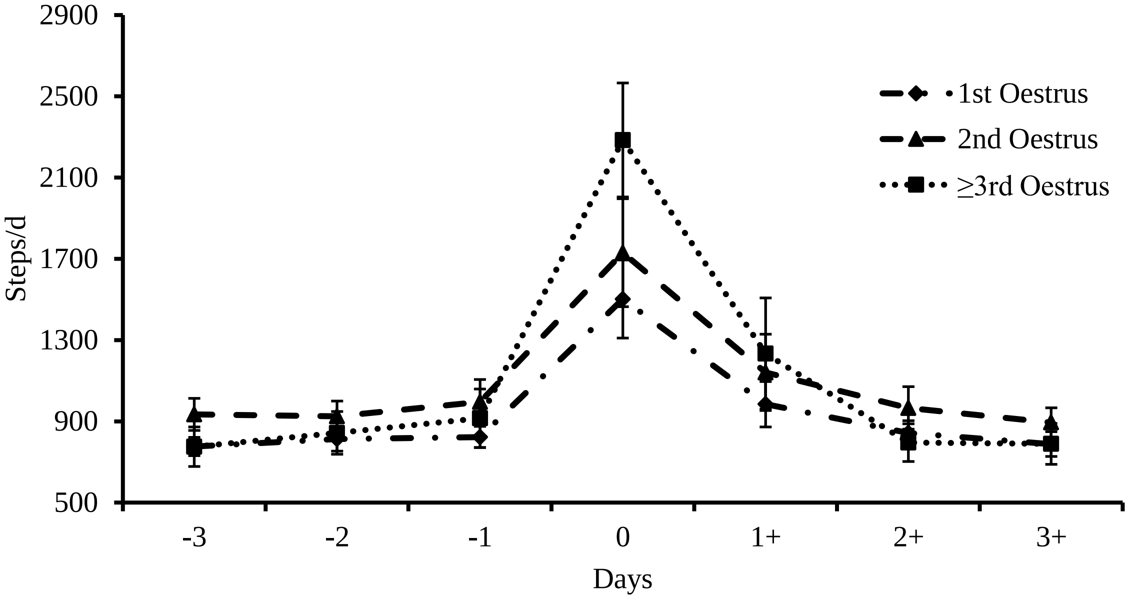

Figure 1: Number of steps 3DB, the day of estrus (0), and 3DA estrus and between first (n=29), second (n=22), and third or more (n=10) postpartum estrus periods.

P-values: E no = 0.444, day = P<0.001, E × d = 0.220. E no=number of estrus postpartum, 0=day of estrus.

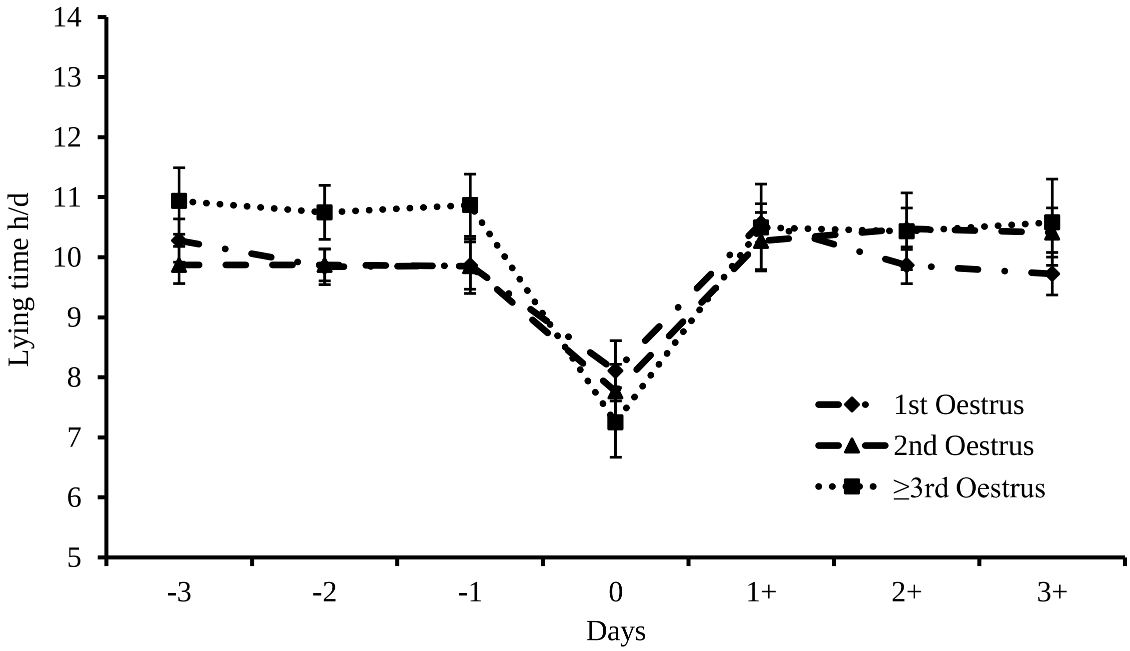

Figure 2: Lying time 3DB, the day of estrus (0), and 3DA estrus and between first (n=29), second (n=22), and third or more (n=10) postpartum estrus periods.

P-values: E no = 0.620, day = P<0.001, E × d = 0.440. E no=number of estrus postpartum, 0=day of estrus.

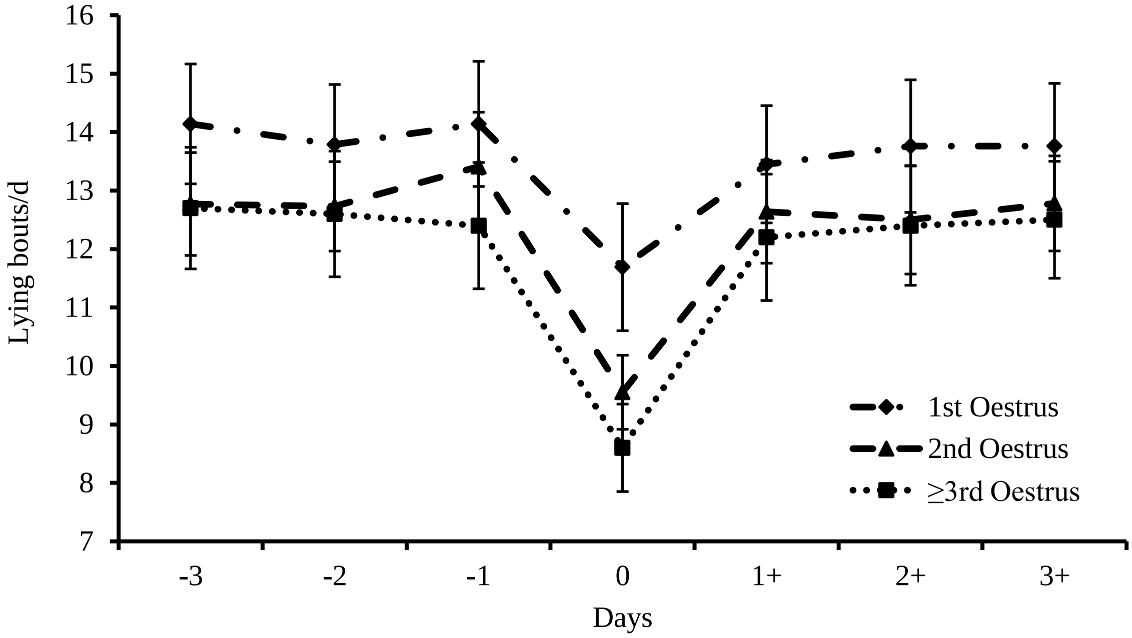

Figure 3: Lying bouts 3DB, the day of estrus (0), and 3DA estrus and between first (n=29), second (n=22), and third or more (n=10) postpartum estrus periods.

P-values: E no = 0.479, day = P<0.001, E × d = 0.567. E no = number of estrus postpartum, 0=day of estrus.

Feeding behavior during the three postpartum estrus periods: There was significant reduction at P<0.001 in dry matter intake (DMI) and feeding duration by the cows on the day of estrus during 1st, 2nd and ≥3rd PP estrus as well as at P≤0.049 in the number of feed visits on estrus day compared to the 3 days before and after. There were no significant differences (P=0.485) in day of estrus on DMI (19.9 ± 0.5, 20.0 ± 0.6, and 20.7 ± 0.6kg/d), feeding duration (2.7 ± 0.2, 2.7 ± 0.2, and 2.7 ± 0.2 h/d), and feed visits (25.2 ± 1.4, 25.6 ± 1.8, and 26.6 ± 2.1 visits/d) between first, second, and third or more PP estrus, respectively (Table 1).

Similarly, a previous study on 20 Friesian cows housed on a farm with a straw yard found them spending significantly less time feeding on the day of estrus (25). Another study also found that lactating cows ate less feed and spent less time feeding on the day of estrus by 10.3% and 20.8%, respectively (28). This decrease in feed intake on the day of estrus may be due to restlessness and higher physical activity during the period (16). This study found that steps taken on the day of estrus was significantly negatively (P<0.001) related with DMI (y = -0.0014 + 22.5; r^2=0.459) on the day of estrus.

Behavioral expressions during the different postpartum estrus periods: The lactating cows showed greater (P=0.002) restlessness during second (87.5%) and third or more (93.8%) PP estrus periods than in the first (Table 2). This agrees with Hurnik et al. (16) in a study conducted at 80 days PP on lactating cows housed in cubicles and that by advancing the number of PP estrus, the estrus activity behavior increases. On the day of ≥3rd PP estrus more cows were seen standing to be mounted (P = 0.013; 75%) than on the 2nd (56.3%) and first (25%) PP estrus periods. This study showed that a higher percentage of estrus cows at third or more estrus stood for mounting compared to the other two PP estrus periods. This result agrees with studies on dairy cows housed continuously (24).

Meanwhile, the cows displayed no significant different in the expression of flehmen behavior (P=0.655), vulva mucous discharge (P=0.367), mounting but not standing (P=0.264), and head to head mounting (P=0.448) over the three PP estrus periods. Moreover, all equally exhibited behaviors of resting the chin on the back of another cow, sniffing the vulva of other cows, and mounting or attempting to mount other cows on the day of estrus. Kyle et al. (20) reported that there were unclear behavioral signs of estrus at the first PP estrus in lactating cows. This may be due to the fetal origin producing high E2 concentrations at the late stage of pregnancy, leading to refractoriness of the hypothalamus to estrogen hormone concentration at the 1st estrus post-partum as noted by Boer et al. (3). Alternatively, the fewer signs of estrous expression before ovulation at the 1st PP may be due to the E2 concentrations in the blood. Forde et al. (12) illustrated that the low LH pulse frequency across the initial estrus post-partum is accompanied by a reduction in ovary follicular development. Boer et al. (3) also found the developed follicles to be a dominant and may not produce adequate E2 to stimulate the typical signs of behavioral estrous expression.

Frequency of expression of behavioral estrus signs during the three postpartum estrus periods: The cows exhibited no differences (P=0.184) in the frequency of the secondary signs of behavioral estrus such as resting the chin on the back of another cow (P=0.162), sniffing vulvas, and mounting or attempting to mount other cows (P=0.218) over the different PP estrus periods (Table 3). Further, they showed no significant (P=0.104) changes in the frequency of the number of total social interactions during the three PP estrus phases although there was (P=0.071) increased licking frequency during the third or more PP estrus period. There were also no significant (P=0.248) changes in the frequency of caressing manifestation during the three phases. These results are inconsistent with Roelofs et al. (30) who noted no significant changes in the secondary estrous signs at 3h-interval observations in lactating cows. Furthermore, similar to the findings of this study, no significant changes in secondary estrous behavioral signs were seen in a study conducted on lactating dairy cows (35 and 36).

There was a higher (P=0.010) expression of butting of other cows in the third or more PP estrus compared to the first and second as well as (P<0.001) udder sniffing for that period (2.8±0.8) compared to the first (0.5±0.3) or second (0.8±0.4). The cows showed higher (P=0.016) frequency of total agonistic expression interaction behaviors in the third or more (10.8±1.9) PP estrus than in the first (6.5±1.0) or second (6.1±0.7). In a study on a synchronized dairy, Kerbrat and Disenhaus (18) found similar frequencies of social expression and agnostic interactions. This study revealed that the frequency of total of agonistic interactions expression was significantly higher in the $\geq 3^{rd}$ PP estrus compared to the other two periods.

Table 1: Dry matter intake, feeding duration, and feed visits 3DB, day of estrus (0), and 3DA and between.

Feeding behavior	Oe No.	Days from estrus							SED		P-value	
		-3	-2	-1	0	1+	2+	3+		E no.	Days	E no. x Days
DMI (kg/d)	1st E	22.0	21.8	22.0	19.9	22.1	21.8	21.6	0.612	0.485	<0.001	0.184
	2nd E	21.8	22.4	21.8	20.0	22.2	22.8	22.9				
	≥3rd E	24.1	22.0	23.7	20.7	22.8	22.3	22.9				
Feeding duration (h/d)	1st E	3.5	3.2	3.3	2.7	3.2	3.1	3.4	0.415	0.847	<0.001	0.390
	2nd E	3.1	3.4	3.5	2.7	3.4	3.6	3.2				
	≥3rd E	3.8	3.7	3.3	2.7	3.4	3.3	3.3				
Feed visits/d	1st E	28.1	26.7	26.2	25.2	27.0	27.6	29.2	4.958	0.756	0.049	0.243
	2nd E	28.8	30.3	29.8	25.6	31.6	30.5	28.5				
	≥3rd E	29.9	27.2	27.9	26.6	27.3	27.5	28.8				

first (n=29), second (n=22) and third or more (n=10) postpartum estrus periods.

Table 2: Expression of signs during behavioral estrus of 1st (n=16), 2nd (n=15) and ≥3rd (n=8) postpartum estrus periods.

Behavioural expression (0/1)	PP estrus			P value
	1st E	2nd E	≥3rd E	
Flehmen	5/16 (31.3%)	4/16 (25%)	3/8 (37.5%)	0.655
Mucous discharge from the vulva	2/16 (12.5%)	4/16 (25%)	3/8 (37.5%)	0.367
Restlessness	10/16 (62.5%)	14/16 (87.5%)	15/8 (93.8%)	0.002
Mounting but not standing	9/16 (56.3%)	10/16 (62.5%)	7/8 (87.5%)	0.264
Mounting or attempting to mount head side other cows	10/16 (62.5%)	11/16 (68.8%)	7/8 (87.5%)	0.448
Standing to be mounted	4/16 (25%)	9/16 (56.3%)	6/8 (75%)	0.013
Sniffing the vulva of another cow	16/16 (100%)	16/16 (100%)	8/8 (100%)	N/A
Chin resting on the back of another cow	16/16 (100%)	16/16 (100%)	8/8 (100%)	N/A
Mounting or attempt to mount other cows	16/16 (100%)	16/16 (100%)	8/8 (100%)	N/A

E= estrus; 1st E=first PP estrus; 2nd E=second PP estrus; and ≥3rd E=third or more PP estrus.

Table 3: Frequency of behavioral estrous signs during 1st (n=16), 2nd (n=15) and ≥3rd (n=8) postpartum estrus periods.

Behavioral estrus signs	PP Estrus			*P* value
	1st E	2nd E	≥3rd E	
Sexual Interaction				
Vulva sniffing of another cow	19.6±5.2	28.3±7.3	34.6±7.4	0.184
Resting chin on the back of another cow	12.9±3.8	23.6±6.8	21.9±3.3	0.162
Mounting or attempt to mount other cows	5.8±1.8	8.1±2.0	10.4±2.1	0.218
Social Interaction				
Caressing	2.6±0.4	2.4±0.6	4.4±1.6	0.248
Licking	$4.8±0.8^{b}$	$3.7±0.6^{b}$	$6.2±1.1^{a}$	0.071
Total Social Interaction	9±1.1	6.1±1.1	10.5±2.6	0.104
Agonistic Interactions				
Head-to-head butting	3.7±0.8	2.8±0.5	4.5±1.0	0.129
Butting of other cows	$2.3±0.4^{b}$	$2.3±0.3^{b}$	$3.5±0.8^{a}$	0.010
Sniffing the udder	$0.5±0.2^{b}$	$1.0±0.3^{b}$	$2.8±0.8^{a}$	0.001
Total Agonistic Interaction	$6.5±1.0^{b}$	$6.1±0.7^{b}$	$10.8±1.9^{a}$	0.016

Means within rows with different superscript letters are significantly different ($P \leq 0.05$).
E= estrus; 1st E=first PP estrus; 2nd E=second PP estrus; and ≥3rd E=third or more PP estrus.

Conclusions

On the day of estrus, high-lactating Holstein Friesian dairy cows housed in cubicles spend more time walking, less time lying down, and have fewer lying bouts. In addition, during the day of estrus, there was lower DMI intake, less feeding durations, and reduced feed visits. This study found that the proportion of observed estrus was higher with increasing postpartum estrus numbers, and that estrus behavior and activity increased while silent estrus decreased.

Supplementary Materials:

No Supplementary Materials.

Author Contributions:

All authors participated in the methodology, writing-original draft preparation, writing-review and editing and agreed to the published version of the manuscript.

Funding:

This research received no external funding.

Institutional Review Board Statement:

The study was conducted following the protocol authorized by the Head of the Ethics Committee, Harper Adams University dairy unit farm, Newport, Shropshire, TF10, 8NB, UK.

Informed Consent Statement:

No Informed Consent Statement.

Data Availability Statement:

No Data Availability Statement.

Conflicts of Interest:

The authors declare no conflict of interest.

Acknowledgments:

The study was financially supported by the Higher Committee of Education Development in Iraq (HCED-Iraq). Special thanks are due to the farm staff of the Harper Adams University (HAU) and technical staff (Carrie Gauld and Sarah Williams) for their support and assistance.

Disclaimer/Journal's Note:

The statements, opinions, and data contained in all publications are solely those of the individual author(s) and contributor(s) and not of AJAS and/or the editor(s). AJAS and/or the editor(s) disclaim responsibility for any injury to people or property resulting from any ideas, methods, instructions, or products referred to in the content.

References

1. Allrich, R. D. (1994). Endocrine and neural control of estrus in dairy cows. Journal of dairy science, 77(9): 2738-2744. https://doi.org/10.3168/jds.S0022-0302(94)77216-7.
2. Al-Mohammedy, A. M. F., and Abdul-Rahaman, Y. T. (2024). The impact of biotin and hcg on the levels of estradiol-17β, progesterone and sheep pregnancy associated plasma protein B (PAPPB) in local Iraqi ewes. Anbar Journal of Agricultural Sciences, 22(1): 106-114. https://doi.org/10.32649/ajas.2024.183704.
3. Boer, H. M. T., Veerkamp, R. F., Beerda, B., and Woelders, H. (2010). Estrous behavior in dairy cows: identification of underlying mechanisms and gene functions. Animal, 4(3): 446-453. https://doi.org/10.1017/S1751731109991169.
4. Butler, W. R. (2003). Energy balance relationships with follicular development, ovulation and fertility in postpartum dairy cows. Livestock production science, 83(2-3): 211-218. https://doi.org/10.1016/S0301-6226(03)00112-X.
5. Chapinal, N., De Passille, A. M., Weary, D. M., Von Keyserlingk, M. A. G., and Rushen, J. (2009). Using gait score, walking speed, and lying behavior to detect hoof lesions in dairy cows. Journal of dairy science, 92(9): 4365-4374. https://doi.org/10.3168/jds.2009-2115.
6. Dalton, J. C., Nadir, S., Bame, J. H., Noftsinger, M., Nebel, R. L., and Saacke, R. G. (2001). Effect of time of insemination on number of accessory sperm, fertilization rate, and embryo quality in nonlactating dairy cattle. Journal of dairy science, 84(11): 2413-2418. https://doi.org/10.3168/jds.S0022-0302(01)74690-5.
7. Dobson, H., Walker, S. L., Morris, M. J., Routly, J. E., and Smith, R. F. (2008). Why is it getting more difficult to successfully artificially inseminate dairy cows?. Animal, 2(8): 1104-1111. https://doi.org/10.1017/S175173110800236X.
8. Dolecheck, K. A. (2015). Assessment of the technical and economic potential of automated estrus detection technologies for dairy cattle.
9. Dolecheck, K. A., Silvia, W. J., Heersche Jr, G., Chang, Y. M., Ray, D. L., Stone, A. E., ... and Bewley, J. M. (2015). Behavioral and physiological changes around estrus events identified using multiple automated monitoring technologies. Journal of dairy science, 98(12): 8723-8731. https://doi.org/10.3168/jds.2015-9645.
10. Domènech, A., Pich, S., Arís, A., Plasencia, C., Bach, A., and Serrano, A. (2011). Heat identification by 17β-estradiol and progesterone quantification in individual

raw milk samples by enzyme immunoassay. Electronic Journal of Biotechnology, 14(4). DOI: 10.2225/vol14-issue4-fulltext-6.

11. Firk, R., Stamer, E., Junge, W., and Krieter, J. (2002). Systematic effects on activity, milk yield, milk flow rate and electrical conductivity. Archives Animal Breeding 45(3): 213-222. https://doi.org/10.5194/aab-45-213-2002.
12. Forde, N., Beltman, M. E., Lonergan, P., Diskin, M., Roche, J. F., and Crowe, M. A. (2011). Oestrous cycles in Bos taurus cattle. Animal reproduction science, 124(3-4): 163-169. https://doi.org/10.1016/j.anireprosci.2010.08.025.
13. Fricke, P. M., Carvalho, P. D., Giordano, J. O., Valenza, A., Lopes Jr, G., and Amundson, M. C. (2014). Expression and detection of estrus in dairy cows: the role of new technologies. Animal, 8(s1): 134-143. https://doi.org/10.1017/S1751731114000299.
14. Gordon, P. (2011). Oestrus detection in dairy cattle. In Practice, 33(10): 542-546. https://doi.org/10.1136/inp.d7479.
15. Hanks, J., and Kossaibati, M. (2012). Key Performance Indicators for the UK national dairy herd. A study of herd performance in, 500: 1-26.
16. Hurnik, J. F., King, G. J., and Robertson, H. A. (1975). Estrous and related behaviour in postpartum Holstein cows. Applied Animal Ethology, 2(1): 55-68. https://doi.org/10.1016/0304-3762(75)90065-6.
17. Isobe, N., Yoshimura, T., Yoshida, C., and Nakao, T. (2004). Incidence of silent ovulation in dairy cows during postpartum period. Deutsche Tierarztliche Wochenschrift, 111(1): 35-37.
18. Jónsson, R., Blanke, M., Poulsen, N. K., Caponetti, F., and Højsgaard, S. (2011). Oestrus detection in dairy cows from activity and lying data using on-line individual models. Computers and electronics in agriculture, 76(1): 6-15. https://doi.org/10.1016/j.compag.2010.12.014.
19. Kerbrat, S., and Disenhaus, C. (2004). A proposition for an updated behavioural characterisation of the oestrus period in dairy cows. Applied Animal Behaviour Science, 87(3-4): 223-238. https://doi.org/10.1016/j.applanim.2003.12.001.
20. Kyle, S. D., Callahan, C. J., and Allrich, R. D. (1992). Effect of progesterone on the expression of estrus at the first postpartum ovulation in dairy cattle. Journal of dairy science, 75(6): 1456-1460. https://doi.org/10.3168/jds.S0022-0302(92)77901-6.
21. López-Gatius, F., Santolaria, P., Mundet, I., and Yániz, J. L. (2005). Walking activity at estrus and subsequent fertility in dairy cows. Theriogenology, 63(5): 1419-1429. https://doi.org/10.1016/j.theriogenology.2004.07.007.
22. Lucy, M. C. (2001). Reproductive loss in high-producing dairy cattle: where will it end?. Journal of dairy science, 84(6): 1277-1293. https://doi.org/10.3168/jds.S0022-0302(01)70158-0.
23. Madureira, A. M. L., Silper, B. F., Burnett, T. A., Polsky, L., Cruppe, L. H., Veira, D. M., ... and Cerri, R. L. A. (2015). Factors affecting expression of estrus measured by activity monitors and conception risk of lactating dairy cows. Journal of Dairy Science, 98(10): 7003-7014. https://doi.org/10.3168/jds.2015-9672.
24. Palmer, M. A., Olmos, G., Boyle, L. A., and Mee, J. F. (2012). A comparison of the estrous behavior of Holstein-Friesian cows when cubicle-housed and at

pasture. Theriogenology, 77(2): 382-388. https://doi.org/10.1016/j.theriogenology.2011.08.010.

25. Phillips, C. J. C., and Schofield, S. A. (1990). The effect of environment and stage of the oestrous cycle on the behaviour of dairy cows. Applied Animal Behaviour Science, 27(1-2): 21-31. https://doi.org/10.1016/0168-1591(90)90004-W.
26. Ranasinghe, R. M. S. B. K., Nakao, T., Yamada, K., and Koike, K. (2010). Silent ovulation, based on walking activity and milk progesterone concentrations, in Holstein cows housed in a free-stall barn. Theriogenology, 73(7): 942-949. https://doi.org/10.1016/j.theriogenology.2009.11.021.
27. Reith, S., and Hoy, S. (2012). Relationship between daily rumination time and estrus of dairy cows. Journal of Dairy Science, 95(11): 6416-6420. https://doi.org/10.3168/jds.2012-5316.
28. Reith, S. R. (2016). Influence of estrus on rumination, activity, feed and water intake of dairy cows.
29. Roelofs, J., Lopez-Gatius, F., Hunter, R. H. F., Van Eerdenburg, F. J. C. M., and Hanzen, C. H. (2010). When is a cow in estrus? Clinical and practical aspects. Theriogenology, 74(3): 327-344. https://doi.org/10.1016/j.theriogenology.2010.02.016.
30. Roelofs, J. B., Van Eerdenburg, F. J. C. M., Soede, N. M., and Kemp, B. (2005). Various behavioral signs of estrous and their relationship with time of ovulation in dairy cattle. Theriogenology, 63(5): 1366-1377. https://doi.org/10.1016/j.theriogenology.2004.07.009.
31. Royal, M. D., Darwash, A. O., Flint, A. P. F., Webb, R., Woolliams, J. A., and Lamming, G. E. (2000). Declining fertility in dairy cattle: changes in traditional and endocrine parameters of fertility. Animal science, 70(3): 487-501. https://doi.org/10.1017/S1357729800051845.
32. Shareef, M. A., Essa, A. H., and Saeed, O. A. (2023). Estrus synchronization applications utilized in ruminant animals: A review. Anbar Journal Agricultural Sciences, 21(2): 419-427. https://doi.org/10.32649/ajas.2024.145165.1102.
33. Shipka, M. P. (2000). A note on silent ovulation identified by using radiotelemetry for estrous detection. Applied Animal Behaviour Science, 66(1-2): 153-159. https://doi.org/10.1016/S0168-1591(99)00079-9.
34. Sveberg, G., Refsdal, A. O., Erhard, H. W., Kommisrud, E., Aldrin, M., Tvete, I. F., ... and Ropstad, E. (2011). Behavior of lactating Holstein-Friesian cows during spontaneous cycles of estrus. Journal of dairy science, 94(3): 1289-1301. https://doi.org/10.3168/jds.2010-3570.
35. Van Eerdenburg, F. J. C. M., Karthaus, D., Taverne, M. A. M., Mercis, I., and Szenci, O. (2002). The relationship between estrous behavioral score and time of ovulation in dairy cattle. Journal of Dairy Science, 85(5): 1150-1156. https://doi.org/10.3168/jds.S0022-0302(02)74177-5.
36. Walker, W. L., Nebel, R. L., and McGilliard, M. L. (1996). Time of ovulation relative to mounting activity in dairy cattle. Journal of dairy science, 79(9): 1555-1561. https://doi.org/10.3168/jds.S0022-0302(96)76517-7.

37. Walsh, S. W., Williams, E. J., and Evans, A. C. O. (2011). A review of the causes of poor fertility in high milk producing dairy cows. Animal reproduction science, 123(3-4): 127-138. https://doi.org/10.1016/j.anireprosci.2010.12.001.
38. Yániz, J., López-Gatius, F., Bech-Sàbat, G., García-Ispierto, I., Serrano, B., and Santolaria, P. (2008). Relationships between milk production, ovarian function and fertility in high-producing dairy herds in north-eastern Spain. Reproduction in Domestic Animals, 43: 38-43. https://doi.org/10.1111/j.1439-0531.2008.01227.x.
39. Yániz, J. L., Santolaria, P., Giribet, A., and López-Gatius, F. (2006). Factors affecting walking activity at estrus during postpartum period and subsequent fertility in dairy cows. Theriogenology, 66(8): 1943-1950. https://doi.org/10.1016/j.theriogenology.2006.05.013.
40. Zebari, H. M., Rutter, S. M., and Bleach, E. C. (2018). Characterizing changes in activity and feeding behaviour of lactating dairy cows during behavioural and silent oestrus. Applied Animal Behaviour Science, 206: 12-17. https://doi.org/10.1016/j.applanim.2018.06.002.
41. Zebari, H. M., Rutter, S. M., and Bleach, E. C. (2019). Fatty acid profile of milk for determining reproductive status in lactating Holstein Friesian cows. Animal Reproduction Science, 202: 26-34. https://doi.org/10.1016/j.anireprosci.2019.01.004.