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Ovarian Recovery and Stress Markers for Lame Dairy Cows During The Transition Period

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Abstract | Lameness is a common and important health problem affecting cattle in dairy farms. It is considered a major factor influencing both productive and reproductive outcomes. Therefore, a randomized clinical trial was conducted at a commercial dairy farm on 300 lactating cows managed under a regular production program. Cows were divided into two categories: non-lame with regular ovarian rebound and lame with delayed ovarian activity, ovarian activity was confirmed by serum progesterone levels. Clinical and reproductive data were recorded, including age, calving date, and reproductive parameters such as postpartum estrus, services per conception, days open, and pregnancy rate. Blood samples were collected and stored for biochemical, hormonal, and molecular assays. The results showed that reproductive performance significantly deteriorated in lame cows compared to the non-lame group. Hormonal assays demonstrated significant increases in cortisol ($p = 0.018$) and significant decreases in progesterone levels ($p = 0.001$) in lame cows compared to healthy ones. Biochemical and molecular assays revealed marked increases in oxidative and inflammatory markers in lame cows relative to healthy cows. Moreover, mRNA expression of steroidogenesis genes was significantly downregulated in affected lame cows with ($p < 0.0001$) in both CYP17A1 and StAR expressions. It can be concluded that lameness negatively affects reproductive performance in dairy cows, as evidenced by impaired fertility indices, hormonal imbalance, and elevated stress, oxidative, and inflammatory markers.

Keywords | Lameness, Dairy, Ovarian activity, Reproductive data, Inflammatory markers

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INTRODUCTION

Reproductive and productive outcomes are crucial for the sustainability of dairy farms (Logroño *et al.*, 2021). Lameness is a major health issue in dairy herds and is considered as the third most significant cause of economic losses in the dairy industry, following mastitis (Sucena Afonso, 2021; Sadiq *et al.*, 2024). It has also been shown to negatively influence reproductive performance (Praxitelous *et al.*, 2023). The timing of postpartum ovarian activity resumption is a key determinant of subsequent fertility in dairy cattle. A delay in ovarian rebound is strongly associated with reduced fertility (Sakaguchi *et al*., 2023). Multiple factors, including nutrition, season, and management practices, have been implicated in influencing postpartum ovarian cyclicity in dairy cows (Sammad *et al*., 2022; Ryder *et al*., 2023; Triwutanon and Rukkwamsuk, 2023).

Hoof disorders are painful conditions that frequently arise during the postpartum period and are recognized as a major factor reducing fertility in dairy cattle. A recent study investigating the association between lameness and delayed ovarian cyclicity within the first 60 days postpartum in Holstein cows reported that lame cows were 3.5 times more likely to experience delayed ovarian cyclicity compared with non-lame cows (Praxitelous *et al*., 2023; Martin, 2024). Earlier findings also demonstrated that lame cows had a markedly lower conception rate at first service (17.5% vs. 42.6%) and a higher prevalence of ovarian cysts (25.0% vs. 11.1%) compared with their non-lame counterparts (Melendez *et al*., 2018).

The precise mechanism linking lameness with delayed ovarian cyclicity is not yet fully understood. Lameness acts as an acute or chronic stressor that adversely affects endocrine function and impairs reproduction through its influence on the hypothalamic-pituitary-adrenal axis (Ward, 2019; Martin, 2024). Activation of adrenocorticotrophic hormone suppresses the pulsatile release of luteinizing hormone, leading to the development of persistent follicles and delayed ovulation (McCosh *et al*., 2019). Similarly, administration of exogenous glucocorticoids has been shown to suppress pituitary gonadotropin secretion, disrupt metabolic signaling, and reduce progesterone production by the corpus luteum (Necula *et al*., 2024). As a result, lame cows experience a delayed resumption of postpartum ovarian cyclicity compared with non-lame cows Guáqueta *et al*. (2014) and Melendez *et al*. (2018), along with a higher incidence of cystic ovarian disease, lower progesterone levels, and increased embryo losses (Praxitelous *et al*., 2023).

Lameness indirectly reduces fertility in dairy cows through its physical limitations (Garvey, 2022). Estrous behavior is less pronounced in lame cows (Morris *et al*., 2011), and they are mounted less frequently and for shorter durations compared with non-lame cows (Van Nuffel *et al*., 2015). A clearer understanding of the mechanisms by which lameness affects fertility is essential for improving the management of reproductive efficiency in affected cattle (Parkinson, 2018). Recognizing the impact of lameness during the early postpartum period on ovulation failure and energy balance in lactating cows may assist clinicians in developing effective prevention and treatment strategies. Therefore, the present study was designed to test the hypothesis that lameness-induced chronic stress disrupts reproductive function via hormonal imbalance, oxidative stress, and inflammatory signaling, and to characterize the expression of key genes involved in this process.

MATERIALS AND METHODS

Farm and animals

A randomized clinical trial was carried out on a commercial Holstein–Friesian dairy farm comprising on 300 lactating cows. The animals were housed in a cubicle (free-stall) barn with 150 mattress-bedded stalls and a slatted floor that was scraped weekly. Cows were offered a total mixed ration, milked three times daily, and bred by artificial insemination. Concentrate intake was increased 4 weeks before calving and peaked 3 weeks after parturition. The average annual milk yield per cow in the herd was 8,500 kg of energy-corrected milk. Routine hoof trimming was performed twice yearly, in autumn and spring, by a professional hoof trimmer assisted by experienced herdsmen. Fresh water was provided ad libitum.

Study design

The animals were grouped to lame and non-lame cows according to locomotion score (1-5) which was carried out by well-trained person, at which score 1 being a normal, healthy cow and score 5 being a severely lame cow that cannot bear weight. Based on the concentration of progesterone in the blood during the postpartum period (ranged from 35 to 45 days after parturition), which is a reliable indicator of the resumption of ovarian activity, the study found that lame cows, as opposed to non-lame cows, had a delayed ovarian cycle and a reduced ovulation rate. This was demonstrated by the lower ($P < 0.05$) progesterone concentration in the blood (Figure 2). To create the next groups, lame non-ovulated (n = 10) and non-lame ovulated (healthy, n = 10), they will split into those who experienced ovulation or not. Further laboratory studies were performed on these cows in order to have a better understanding of the mechanisms behind the effect of lameness on the reproductive state of postpartum dairy cows.

Clinical and reproductive data

Reproductive records were collected from 2 weeks prepartum to 3 months postpartum, including cow identification number, age, calving date, postpartum estrus, services per conception, days open, and pregnancy rate.

Blood sampling and laboratory analyses

Blood samples were collected from the coccygeal vein of the dairy cows into 10-mL Vacutainer tubes. Blood samples were collected in a time ranged from 35 to 45 days after parturition after they were diagnosed as lame cows, in the control healthy cows the blood samples were collected at the corresponding same time after parturition. Each sample was divided into two portions: one portion was preserved with TRIzol and stored at −80°C for RNA extraction and gene expression analysis, while the other was allowed to clot and kept at 4°C until serum separation. Clotted samples were centrifuged at 3000 rpm for 20 minutes, and the obtained serum was stored at −20°C until further analysis.

Biochemical analysis

Hormone assays

Serum hormone concentrations were measured using the colorimetric enzyme-linked immunosorbent assay (ELISA). Progesterone levels were determined according to the method of Aufrère and Benson (1976), while cortisol concentrations were assessed following the procedure described by Ruder *et al.* (1972).

Blood glucose level test

Blood glucose concentrations were measured using a handheld glucometer by immediately applying fresh blood samples to glucose test strips, with readings obtained within a few seconds.

Protein profile assays

Protein profile assays were performed using spectrophotometry with commercial kits to determine total protein (Gornal *et al*., 1949) and albumin (Doumas *et al*., 1971), while globulin levels were calculated.

Lipid peroxidation and antioxidant-dependent biomarkers

Malondialdehyde (MDA) content, an oxidative stress marker, nitric oxide (NO) concentration, and the antioxidant activity of glutathione peroxidase (GPx) were assayed using enzymatic colorimetric techniques with commercial kits, following the methods of Ohkawa *et al.* (1979), Rajaraman *et al.* (1998), and Paglia and Valentine (1967), respectively.

RT-PCR and gene expression

RNA was extracted from blood samples of Holstein dairy cows using the GeneJET Genomic RNA Extraction Kit, following the manufacturer's instructions (Jena Bioscience, Germany). Real time PCR (RT-PCR) was performed to amplify fragments of selected genes using primers designed from published bovine gene sequences available in GenBank. The primer sequences, annealing temperatures, and sizes of the amplified fragments, with GAPDH used as the housekeeping gene, are presented in Table 1. Melt-curve analysis validated the specificity of amplicons, each of which produced a single peak, and PCR efficiency for all primers was found to be between 90 and 110%. Relative gene expression was calculated using the 2−ΔΔCt method (Pfaffl, 2001).

Statistical analysis

Paired comparisons between lame and non-lame cow groups were performed using Student's *t*-test after checking data for normality using the Shapiro-Wilk test. Quantitative data were expressed as the mean ± standard error of the mean (SEM). A *p*-value of < 0.05 was considered statistically significant.

RESULTS

Impact of lameness on reproductive performance

Reproductive performance was significantly affected by lameness in dairy cows, as demonstrated by the analyzed

Table 1: Forward and reverse oligonucleotide-based real-time PCR primers for genes being investigated.

Investigated marker	Primer	Product size (bp)	Annealing temperature (°C)	GenBank isolate
IL-8	F5′- AAGTGGGTGCAGAAGGTTGT -3 R5′-CAACCCTACACCAGACCCAC-3′	187	58	JN559767.1
TLR4	F5′- GGGTGCGGAATGAACTGGTA-3 R5′- TCCTGGATGATATTGGCGGC -3′	115	60	NM_174198.6
TNF-α	F5′- GAAGTTGCTTGTGCCTCAGC -3′ R5′- TGGGGACTGCTCTTCCCTC- 3′	113	60	NM_173966.3
IGF-1	F5′- CCACCCTGACCTGCTGTAAA -3′ R5′- AGAGCATCCACCAACTCAGC- 3′	176	58	XM_005206500.5
DGAT1	F5′- GGTCGCGGCCTTCGAT -3′ R5′- CCACGTCTACGTCTCCGTC - 3′	123	58	NM_174693.2
CYP17A1	F5′- GATCGTGGCCTACCTGCTAC -3′ R5′- CCACAACGTCTGTGCCTTTG - 3′	242	58	NM_174304.3
STAR	F5′- CTGCCCTGCTCTTGAAGCTA -3′ R5′- AGAGCCTTGTCCGCATTCTC - 3′	226	60	NM_174189.3
GAPDH	F5′- AGCCGTAACTTCTGTGCTGT -3′ R5′- TGCCGTGGGTGGAATCATAC - 3′	200	60	NM_001034034.2

parameters (Figure 1A–C). As shown in Figure 1A, lame cows required significantly more services per conception (S/C) compared to non-lame cows (2.6 ±0.21 vs. 1.72 ±0.12, p = 0.021), indicating reduced conception efficiency. Similarly, Figure 1B illustrates that lame cows had asignificantly longer number of days open than non-lame cows (160 ±2.89 days vs. 77.68 ± 0.38 days, p < 0.0001), reflecting a delay in achieving successful pregnancy.Moreover, the pregnancy rate was markedly lower in lame cows compared to non-lame counterparts, as shown in Figure 1C (24.33 ±2.33 % vs. 51.67 ± 7.27 %, p = 0.023), signifying a substantial negative impact of lameness on reproductive success.

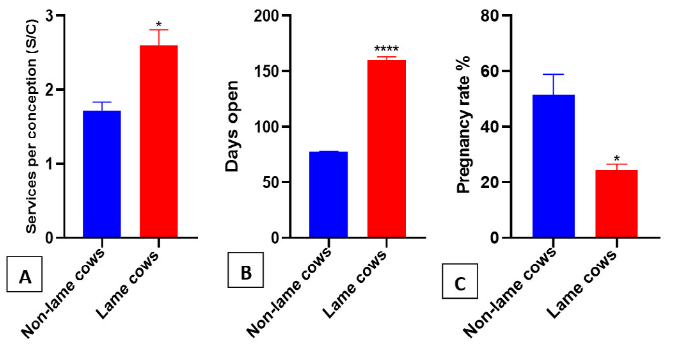

Figure 1: Reproductive parameters; (A) Services per conception (S/C), (B) Days open, and (C) Pregnancy rate % in lame (n = 10) and non-lame (control; n = 10) postpartum dairy cows. All data were analyzed by the student's t-test, *: significant at p < 0.001. ***: significant at p < 0.0001.

Impact of lameness on circulating progesterone and cortisol levels

Lameness in dairy cows significantly affected circulating hormone levels, specifically progesterone and cortisol concentrations (Figure 2A–B). As shown in Figure 2A, lame cows exhibited a significant decrease in serum progesterone levels compared to non-lame cows (0.52 ±0.10 vs. 1.91±0.13 ng/ml), p= 0.001), indicating impaired luteal function and reproductive hormonal imbalance. Conversely, Figure 2B shows that cortisol levels were significantly elevated in lame cows relative to non-lame cows (2.55±0.21 vs. 1.41±0.20 µg/dl, p = 0.018), suggesting a stress-induced endocrine response associated with lameness.

Impact of lameness on metabolic and protein profiles

Lameness in dairy cows induced significant changes in metabolic and protein profiles (Figure 3A–B). As shown in Figure 3A, serum glucose levels were significantly elevated in lame cows compared to non-lame cows (79.33 ± 3.67 vs. 51.33 ± 3.18 mg/dl, p = 0.005), suggesting a stress-induced hyperglycemic response. In terms of protein metabolism (Figure 3B), there was no significant difference in total protein levels between the two groups (8.17 ± 0.20 and 7.73 ± 0.55 g/dl, p = 0.498). However, albumin concentration was significantly reduced in lame cows relative to non-lame cows (2.27 ± 0.32 vs. 3.63 ± 0.09 g/dl, p = 0.014), while globulin concentration was significantly increased (6.17 ± 0.07 vs. 4.77 ± 0.32 g/dl, p = 0.013), indicating a shift in the albumin/globulin ratio that may reflect inflammatory or immune processes.

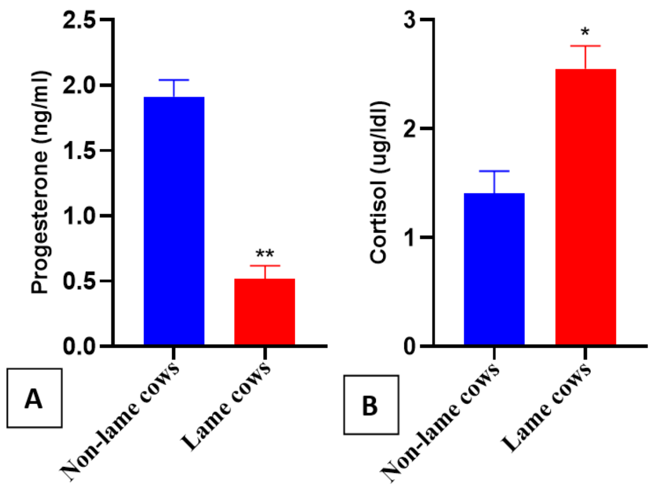

Figure 2: Serum concentration of (A) Progesterone (ng/ml) and (B) Cortisol (µg/dl) in lame (n = 10) and non-lame (control; n = 10) postpartum dairy cows. All data were analyzed by the student's t-test, *: significant at p <0.05. **: significant at p < 0.005

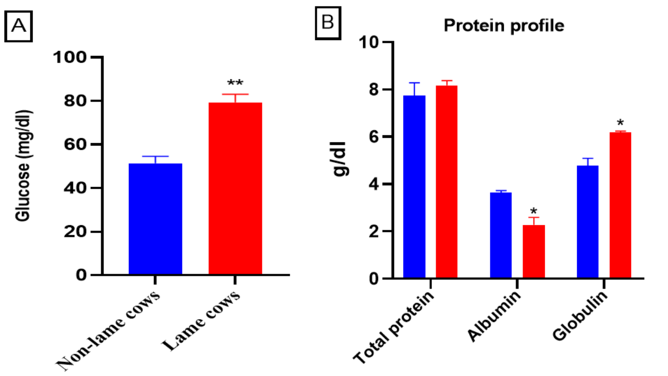

Figure 3: (A) Blood glucose levels (mg/dl) and (B) Serum concentrations of total protein, albumin, and globulin (g/dl) in lame (n = 10) and non-lame (control; n = 10) postpartum dairy cows. All data were analyzed by the student's t-test, *: significant at p < 0.05. **: significant at p < 0.001.

Impact of lameness on oxidative status

Lameness in cows is associated with increased oxidative and nitrosative stress, as well as decreased antioxidant enzyme activity (Figure 4A-C). The present results showed a significant increase (p < 0.05) in MDA (Figure 4A) and

NO (Figure 4B) levels and a significant decrease in serum GPx (Figure 4C) concentration in lame non-ovulated cows compared to non-lame ovulated cows (80.80 ± 2.52 vs. 19.33 ± 3.12 nmol/mL, $p = 0.0001$, 7.50 ± 0.51 vs. 3.43 ± 0.33 µmol/l, $p = 0.003$, and 72.07 ± 4.74 vs. 118.6 ± 4.71 mU/ml, $p = 0.002$, respectively).

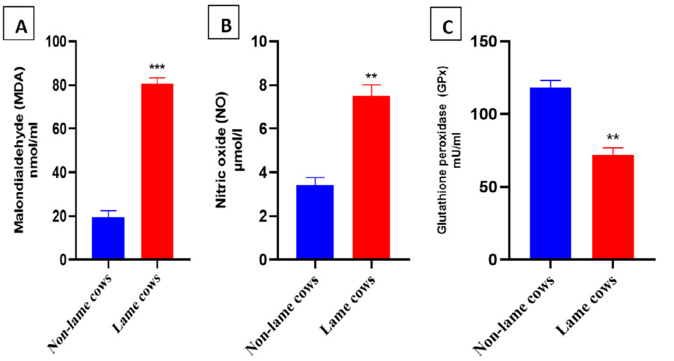

Figure 4: Serum concentrations of (A) Malondialdehyde (MDA) (nmol/ml), (B) Nitric oxide (NO) (µmol/l), and (C) Glutathione peroxidase (GPx) (mU/ml) (n = 10) and non-lame (control; n = 10) postpartum dairy cows. All data were analyzed by the student's t-test, *: significant at $p < 0.05$. **: significant at $p < 0.005$***: significant at $p < 0.0001$

Impact of lameness on steroidogenesis

The mRNA expression levels of CYP17A1 and StAR were significantly reduced in lame cows compared to non-lame controls (Figure 5A–B, respectively). CYP17A1 expression was markedly downregulated in lame cows (~80.2% decrease, $P < 0.05$), suggesting impaired steroidogenesis (Figure 5A). StAR expression showed a significant reduction (~63.8% decrease, $p < 0.01$), indicating diminished cholesterol transport into mitochondria for steroid hormone biosynthesis (Figure 5B).

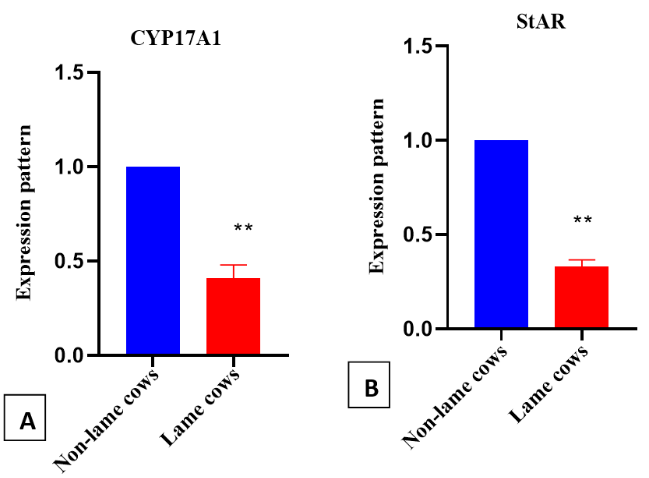

Figure 5: Relative expression of (A) CYP17A1 and (B) StAR in lame (n = 5) and non-lame (control; n = 5) postpartum dairy cows. All data were analyzed by the student's t-test, **: significant at $p < 0.0001$.

Impact of lameness on growth regulation, and lipid metabolism

The mRNA expression levels of IGF-1 and DGAT1 were significantly reduced in lame cows compared to non-lame controls (Figure 6A-B, respectively). IGF-1 mRNA levels were significantly decreased (~62.5% reduction, $p < 0.01$), implying a suppression of growth and metabolic activity associated with lameness (Figure 6A). DGAT1 expression also declined in lame cows (~45.2% decrease, $p < 0.05$), which may reflect altered lipid metabolism or milk fat synthesis under stress or inflammation (Figure 6B)

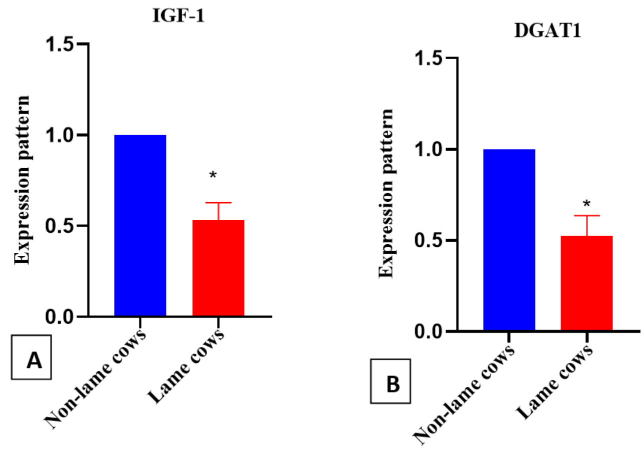

Figure 6: Relative expression of (A) IGF-1 and (B) DGAT1 in lame (n = 5) and non-lame (control; n = 5) postpartum dairy cows. All data were analyzed by the student's t-test, *: significant at $p < 0.01$.

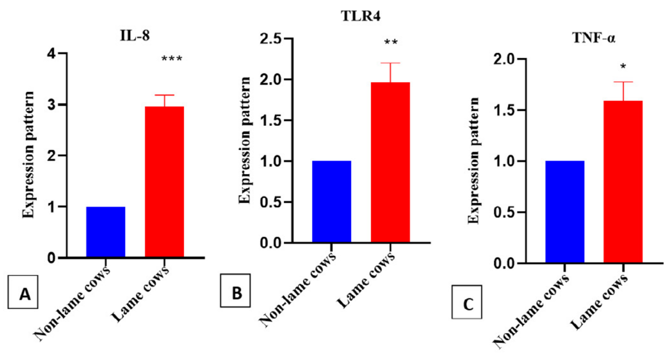

Figure 7: Relative expression of (A) IL-8, (B) TLR4, and (C) TNF-α in lame (n = 5) and non-lame (control; n = 5) postpartum dairy cows. All data were analyzed by the student's t-test, *: significant at $p < 0.05$. , **: significant at $p < 0.02$. , ***: significant at $p < 0.0005$

Impact of lameness on inflammatory response

The expression levels of pro-inflammatory genes IL-8, TLR4, and TNF-α were significantly upregulated in lame cows compared to non-lame controls, indicating an activated inflammatory response (Figure 7A-C, respectively). IL-8 expression showed a significant increase in lame cows, approximately 1.8-fold higher than in non-lame cows ($p < 0.05$). This upregulation reflects enhanced neutrophil recruitment and activation in response to tissue injury and inflammation in lame animals (Figure 7A). TLR4 expression was markedly elevated in lame cows (~2.0-fold increase, $p < 0.01$), suggesting amplified recognition of pathogen-associated molecular patterns and activation of innate immune signaling cascades (Figure 7B). TNF-α, a key pro-inflammatory cytokine, was also significantly upregulated in lame cows (~1.7-fold increase, $p < 0.05$), indicating systemic inflammatory activation possibly contributing to tissue degeneration and pain associated with lameness (Figure 7C).

DISCUSSION

Reproductive and productive outcomes are critical to dairy farms (Logroño *et al*., 2021). Lameness is a major health issue in dairy farms and is considered the third leading cause of economic loss in the dairy industry, after mastitis (Bruijnis *et al*., 2010; Huxley, 2013) and negatively impacting reproductive performance (Praxitelous *et al*., 2023). However, the exact mechanism linking the lameness and delayed ovarian cycle is still not fully understood.

The present study demonstrates that dairy cow lameness contributes to substantial impairments in reproductive performance, as evidenced by increased services per conception, prolonged days open, and reduced pregnancy rate. These findings are consistent with previous research suggesting that lameness represents a significant welfare issue and economic burden in dairy herds, not only due to decreased milk production but also due to its profound effects on fertility (Logroño *et al*., 2021; Tsousis *et al*., 2022). Lame cows required significantly more inseminations to achieve conception compared to non-lame cows. This increase in services per conception likely reflects both physiological and behavioral consequences of lameness, including altered estrous behavior, reduced detection of estrus, and possible disruptions in the hypothalamic–pituitary–ovarian axis due to chronic stress and inflammation (Ward, 2019; Martin, 2024). Additionally, the prolonged days open observed in lame cows highlights a delayed return to reproductive cyclicity and extended inter-calving intervals (Muasa, 2021). Such delays can be attributed to inadequate estrous expression, poor response to breeding, or silent ovulation, which are commonly reported in lame cows suffering from pain and reduced feed intake. Most notably, the significantly reduced pregnancy rate in lame cows underscores the cumulative reproductive burden imposed by lameness. This reduced fertility can have long-term implications for herd productivity and sustainability, necessitating timely detection, effective treatment, and preventive measures for lameness in dairy operations. Overall, these findings emphasize the critical importance of locomotion monitoring and hoof health management as integral components of reproductive management strategies in dairy herds.

Lameness is an acute or chronic stressor that influences endocrine function through the hypothalamus hypophyseal adrenal axis (Necula *et al*., 2024). In the current study, lame cows exhibited markedly reduced serum progesterone levels and elevated cortisol concentrations, indicating a stress-induced hormonal imbalance that may impair reproductive function (Wrzecińska *et al*., 2021). Progesterone is a critical reproductive hormone required for the initiation and continuation of pregnancy (Nagy *et al*., 2021). The significant reduction in progesterone levels observed in lame cows suggests impaired luteal activity, which could compromise ovulation, embryo development, and implantation success (Lonergan *et al*., 2016). This hormonal insufficiency likely contributes to the poorer reproductive outcomes previously described, including increased services per conception, prolonged days open, and reduced pregnancy rate as shown in our study. In contrast, the significantly increased cortisol levels in lame cows reflect an activated hypothalamic–pituitary–adrenal axis response to pain and physiological stress (Brown and Vosloo, 2017). Chronic elevation of cortisol has been shown to exert suppressive effects on gonadotropin-releasing hormone secretion, luteinizing hormone pulsatility, and overall ovarian function (McCosh *et al*., 2019). This stress-mediated disruption may further aggravate reproductive inefficiency in lame animals (Lagoda *et al*., 2022).

Our results are consistent with Morris *et al*. (2011) who noticed lower progesterone levels in milk in lamed cows for 5 successive days prior to prostaglandin administration than healthy cows. In the same line, O'Driscoll *et al*. (2015) reported that lame cows had cortisol levels that were 49% higher than sound one. Also, Radostits *et al*. (2006) observed that cows with ulcers and suffer from lameness exhibited elevated circulating cortisol concentrations compared with sound cows, exceeding the normal range for dairy cattle (4.7–7.6 ng/mL). Contrary to our results, Almeida *et al*. (2008) didn't find significant difference between lame cows and sound cows in serum cortisol levels. The inconsistency may stem from the extent and persistence of lameness, timing of sample collection relative to pain episodes, sample size, or individual variability in stress resilience (Merridale *et al*., 2022). Additionally, cortisol levels can be influenced by various confounding factors such as handling, environment, and sampling technique, which may affect the sensitivity of cortisol as a sole indicator of chronic pain or stress (Villafañe *et al*., 2020). Overall, while some variation exists across studies, the weight of evidence, including our findings, supports the association between lameness and altered endocrine function, particularly increased cortisol and decreased progesterone (Tsousis *et* *al*., 2022; Necula *et al*., 2024). These hormonal disruptions likely reflect both the physiological stress and potential impairment of reproductive function in affected animals (Wrzecińska *et al*., 2021), reinforcing the need for early detection and effective management of lameness in dairy herds.

At the gene level, lameness dramatically curtailed transcription of key steroidogenic genes. CYP17A1 mRNA fell by ~80 %, while StAR declined by ~64 %. Both genes are indispensable: StAR translocates cholesterol into mitochondrial membranes, and CYP17A1 catalyzes pivotal 17-α-hydroxylase/17,20-lyase steps that direct progesterone toward androgen and estrogen synthesis (Turcu *et al*., 2020). The downregulation of CYP17A1 and STAR may indicate a disruption in adrenal and gonadal steroid hormone synthesis, which may contribute to poor reproductive performance observed in chronically lame animals (Weller, 2016; Wolfenson *et al*., 2019). Similar repression of StAR and CYP17A1 has been reported in bovine follicular cysts and other steroidogenic disorders, highlighting the sensitivity of these loci to metabolic and inflammatory stress (Lima *et al*., 2019; Xu *et al*., 2023). Glucocorticoid-responsive elements in their promoters are high, this upregulation could potentially be mediated by the heightened cortisol milieu observed in lame cows, thereby compounding the progesterone deficit (Berezowski, 2023). Taken together, our data support a model in which lameness-related pain and inflammation initiate a stress cascade that elevates cortisol, down-regulates steroidogenic gene expression, and ultimately depresses progesterone production. The endocrine disruption documented here provides a mechanistic bridge between claw-health disorders and the well-characterized decline in reproductive performance of lame dairy cows. Routine locomotion scoring, prompt therapeutic hoof care, and effective analgesia are therefore vital not only for welfare but also for maintaining endocrine homeostasis and farm profitability (Edwardes, 2023).

Importantly, these endocrine disruptions appear to be closely linked to the oxidative stress status in lame cows (Tufarelli *et al*., 2023). Previous studies have shown that dairy cows with claw lesions or abnormal gait have more systemic pro-oxidants than healthy cows (Zhao *et al*., 2015; Abuelo *et al*., 2016). MDA, a lipid peroxidation product, has been extensively used as a biomarker of lipid peroxidation and oxidative damage (Jadoon and Malik, 2017) and pain (Herzberg *et al*., 2019). On the other side, serum GSH-Px activity contributes to the oxidative defense of animal tissues by catalyzing the reduction of hydrogen and lipid peroxides (TERZI, 2020). Our study revealed that the lame cows displayed an imbalance in their antioxidant status. Where the lame cows showed a significant rise in MDA and NO activity and a decline in GPx when compared to the non-lame cows. These findings provide strong evidence for the potential role of oxidative damage in the pathogenesis of lameness. The findings of Zhao *et al*. (2015) is consistent with our findings, which showed that the serum of lame cows had elevated MDA levels and decreased SOD activity. Additionally, a rise in MDA concentration was observed in the serum of cows suffering from chronic inflammatory lameness by Al-Qudah and Ismail (2012). In contrast to study findings, Herzberg *et al*. (2019) found no variations in the spinal cord activity of superoxide dismutase, GPx, catalase, and total antioxidant response between lame and non-lame cows. This contrast may be attributed to differences in the biological matrices analyzed. While our study and others focused on systemic (serum) markers of oxidative stress, Herzberg *et al*. (2019) examined localized enzymatic activity within the central nervous system, which may be less sensitive to peripheral inflammatory changes or may have different regulatory mechanisms (Glass *et al*., 2010; Ransohoff and Brown, 2012). Moreover, the chronicity, severity, and etiology of lameness, along with sampling timing, can all influence oxidative stress biomarkers (Al-Qudah and Ismail, 2012; Zhao *et al*., 2015). Our results contribute to the growing body of evidence indicating that oxidative stress plays a central role in the pathophysiology of lameness and its associated systemic consequences (Sadiq *et al*., 2024).

It was documented that there is association between oxidative damage and prolonged pain or inflammation in lame animals (Müller *et al*., 2023). These consistent findings may indicate that systemic oxidative stress is a common consequence of lameness, likely resulting from inflammatory processes, tissue damage, and the physiological stress response (Abuelo *et al*., 2016). Similarly, the expression levels of pro-inflammatory genes IL-8, TLR4, and TNF-α were significantly upregulated in our studied lame cows compared to non-lame controls, indicating an activated inflammatory response. IL-8 expression showed a significant increase in lame cows, approximately 1.8-fold higher than in non-lame cows. This up-regulation reflects an enhanced chemotactic response for neutrophils at inflamed sites (Matsushima *et al*., 2022). TLR4 expression was markedly elevated in lame cows (~2.0-fold increase) (Bhattarai *et al*., 2018). TNF-α, a key pro-inflammatory cytokine, was also significantly upregulated in lame cows (~1.7-fold), indicating systemic inflammatory activation possibly contributing to tissue degeneration and pain associated with lameness (Herzberg *et al*., 2020). These findings collectively confirm the inflammatory nature of lameness, with significant transcriptional activation of immune-related genes in affected animals. The upregulation of TLR4 likely initiates a cascade of immune activation via NF-κB, promoting the transcription of TNF-α and IL-8, which in turn sustains and amplifies inflammation and leukocyte infiltration (Yu *et al*., 2022). This inflammatory loop may contribute to tissue degradation, increased pain sensitivity, and the chronicity of lameness (Herzberg *et al*., 2020). These findings emphasize the need for early anti-inflammatory intervention and provide a potential molecular basis for biomarker development in the diagnosis and management of lameness in dairy cattle.

In addition, lameness is an indirect suppressor of fertility in dairy cows, where it results in less time of feeding (Logroño *et al*., 2021) and less dry matter intake (Garvey, 2022). The current results indicate that lameness in dairy cows is associated with significant metabolic and biochemical alterations, as evidenced by increased glucose levels and disturbances in serum protein fractions. These findings further support the systemic impact of lameness beyond localized pain or locomotor impairment (Urban-Chmiel *et al*., 2024). The elevated serum glucose levels observed in lame cows may reflect a physiological stress response driven by increased cortisol secretion, as confirmed in our earlier hormonal findings (Necula *et al*., 2024). Cortisol promotes gluconeogenesis and inhibits peripheral glucose uptake, leading to hyperglycemia (Janssen, 2022). This adaptive mechanism, while beneficial in acute stress, can become detrimental during chronic conditions such as lameness, where sustained hyperglycemia may impair immune function and reproductive efficiency (Endris and Feki, 2021). On the same line, Habel and Sundrum (2023) found elevated levels of glucose in one third of cows affected by sole ulcers. Also, Cucunubo Santos *et al*. (2022) noticed that lame cow tended to have higher glucose levels than controls. Contradicted to our results, Cucunubo Santos *et al*. (2022) reported subclinical hypoglycemia in lame cows at day 60, this contrast may be due to the time of sampling in our study, sampling was done at 35-45 days after parturition.

The altered protein profile also provides important insights into the systemic effects of lameness (Necula *et al*., 2024). Although total protein levels did not differ significantly, lame cows exhibited a significant decrease in albumin levels and a corresponding increase in globulin concentrations. It is known that lame cows have less time to feed and may exhibit changes in feeding behavior (Logroño *et al*., 2021); the duration of lameness seems, in our study, to be too short to affect total protein in the body. Albumin, a negative acute-phase protein, often decreases in response to inflammation or chronic disease (Eckart *et al*., 2020). Conversely, increased globulin levels likely reflect an upregulated immune response or chronic inflammatory state, possibly associated with infectious or traumatic causes of lameness (Michalska *et al*., 2020). The shift in the albumin-to-globulin ratio observed in lame cows is consistent with an inflammatory or catabolic state (Cattaneo *et al*., 2021). This pattern has been previously associated with reduced productivity, delayed healing, and compromised reproductive outcomes, underscoring the importance of early detection and intervention in lame animals. Corresponding toCucunubo Santos *et al*. (2022) reported lower levels of albumin in lame Holestin x Gir cows and recorded non-significant difference in TP levels. Conversely, Bobbo *et al*. (2017) reported that clinically lame cows had numerically higher total serum protein levels than healthy cows. As well, O'Driscoll *et al*. (2015) detected higher levels of protein in lame cows than healthy ones. Overall, these metabolic and protein profile disturbances reinforce the view that lameness is a complex, systemic condition with far-reaching consequences on the health, productivity, and welfare of dairy cows.

In the same line, the present study demonstrated a significant reduction in the expression of genes involved in growth signaling (IGF-1), and lipid metabolism (DGAT1) in lame cows. IGF-1, a key mediator of growth, metabolism, and immune modulation (Yan and Charles, 2018), was significantly reduced (~63% decrease). This decline may reflect impaired somatotropic axis activity and that chronic stress and systemic inflammation associated with lameness suppress hepatic IGF-1 production (Witkowska-Sędek and Pyrżak, 2020). IGF-1 down regulation may contribute to delayed tissue repair, muscle catabolism, and reproductive inefficiency observed in lame animals (Elsayed *et al*., 2019). Moreover, DGAT1 expression was significantly lower in lame cows (~45% reduction). DGAT1 is a key enzyme in triglyceride synthesis and energy storage, and its downregulation indicates disrupted lipid metabolism (Chitraju *et al*., 2019). In dairy cows, reduced DGAT1 activity may result in lower milk fat content and poor energy balance, further aggravating the negative energy status often seen in chronically lame individuals (Ma *et al*., 2022; Barcarolo *et al*., 2024). The metabolic burden imposed by inflammation and oxidative stress, evidenced by increased MDA and NO levels and reduced GPx activity, may directly impair DGAT1-mediated fat synthesis, exacerbating energy deficits during lactation (Masenga *et al*., 2023). Collectively, our findings illustrate the complex interplay between pain, inflammation, oxidative stress, hormonal imbalance, and metabolic disruption in lame dairy cows. The integration of clinical performance, biochemical markers, and gene expression data offers a comprehensive pathophysiological model that explains how lameness leads to poor reproductive and productive outcomes. These results underscore the need for early diagnosis, effective pain and inflammation management, and nutritional interventions, not only to improve animal welfare but also to preserve fertility, productivity, and economic sustainability in dairy herds.

CONCLUSION AND RECOMMENDATIONS

This study demonstrates that lameness in dairy cows is a complex systemic condition that significantly impairs reproductive performance through hormonal imbalances, notably reduced progesterone and elevated cortisol. These disruptions are linked to down regulated steroidogenic genes (StAR, CYP17A1), heightened oxidative stress (MDA, NO), and increased inflammatory markers (IL-8, TLR4, TNF-α). Additionally, lameness alters metabolic health, as seen in hyperglycemia, protein profile shifts, and decreased expression of IGF-1 and DGAT1, indicating compromised growth, energy balance, and milk production. Overall, this study provides compelling evidence that lameness in dairy cows is not merely a localized musculoskeletal issue but a multifactorial condition with profound systemic repercussions, affecting endocrine, immune, and metabolic health. The integration of clinical, biochemical, and molecular data in this study provides a comprehensive understanding of the pathophysiology of lameness and its far-reaching consequences. Further investigations may be needed with a greater number of cows under investigation, more sampling plan and anti-inflammatory or antioxidant drugs may be used. Effective lameness prevention, early diagnosis, pain management, and metabolic support are therefore critical not only for animal welfare but also for ensuring reproductive success and economic sustainability in dairy herds.

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NOVELTY STATEMENT

This study reveals novel evidence that lameness impairs reproductive performance in dairy cows through hormonal imbalance, oxidative stress, systemic inflammation and altered steroidogenesis gene expression, highlighting underlying molecular and biochemical mechanisms beyond clinical fertility outcomes.

AUTHOR'S CONTRIBUTION

Dr. Ahmed Ibrahim Elmahdy: Conceptualization, study design, animal management, clinical data collection, and manuscript drafting. Dr. Eman Mohamed Embaby: Laboratory experiments, methodology, statistical analysis, data interpretation and manuscript preparation. Dr. Sherine Mohamed Abbas: Critical review, and revision of the manuscript. Prof. Dr. Ahmed Ateya: Molecular analysis, Data analysis and manuscript editing. Prof. Dr. Youssef Yahia Elseady: Conceptualization, study design, supervision, language revision and final manuscript review.

Ethical approval

All experimental procedures were reviewed and approved by the Animal Care and Use Committee of Mansoura University (MU-ACUC; Approval Code: VM.R.24.11.196). The study was conducted in accordance with the institutional guidelines for animal care and use, and in compliance with internationally accepted standards, including the ARRIVE guidelines and the principles of the NIH Guide for the Care and Use of Laboratory Animals as well as the EU Directive 2010/63/EU on the protection of animals used for scientific purposes.

Generative AI and AI-assisted technology statement

All authors of this work declare that generative AI technologies including large language models (e.g., ChatGPT, Copilot) and text-to-image generators were not utilized in any capacity during the preparation, writing, or editing of this manuscript.

Conflict of interest

The authors have declared no conflict of interest.

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