

# Chronic stress, hormone profiles and estrus intensity in dairy cattle

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## Abstract

The objectives of the present study were to determine if lameness, a model for a natural chronic stressor, affects hormone concentrations in milk prior to estrus and/or the subsequent expression of estrus in the postpartum period. Dairy cows >20 days postpartum were scored for lameness and observed for estrus intensity using a weighted scoring system (>100 points=estrus=Day 0). Increasing lameness score was not associated with daily profiles of milk progesterone (throughout Days –18 to 0), estradiol (Days –6 to 2) or cortisol (Days –18 to 2) around estrus, maximum estradiol values or estradiol concentrations on Day 0. However, *post hoc* pair wise comparisons revealed that prior to estrus, severely lame cows had lower maximum progesterone concentrations compared to nonlame cows ( $1.3 \pm 0.1$ ,  $1.2 \pm 0.2$ ,  $0.7 \pm 0.1$  ng/ml milk;  $P=0.042$ ). Furthermore, severely lame cows expressed behavioral estrus with lower intensity ( $284 \pm 128$  points,  $n=9$ ) compared to moderately lame ( $662 \pm 310$  points,  $n=9$ ) or nonlame animals ( $583 \pm 275$  points,  $n=18$ ;  $P=0.05$  and  $P=0.02$ , respectively). Resting concentrations of cortisol (Days 20–80 postpartum) did not vary between days postpartum or lameness score. The incidence of behavioral estrus was not affected by increasing lameness score, as 54.2%, 56.2% and 50.0% periods with low progesterone were associated with spontaneous estrus expression, respectively. Concluding, in this biological model of chronic stress, lameness did not affect the incidence of behavioral estrus but did reduce estrus intensity once ovarian cyclicity had resumed after calving. This reduced intensity of estrus was associated with lower maximum progesterone values prior to estrus but not abnormal daily cortisol or estradiol values in milk.

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## Introduction

Acute stress has detrimental effects on ovarian cyclicity by disrupting the normal release of hormones from the hypothalamus-pituitary-ovarian axis that normally control reproduction (Dobson et al., 2003; Smith et al., 2003). This disruption, resulting in suppression of pulsatile release of luteinising hormone from the pituitary, may impact on ovarian progesterone and estradiol concentrations, hormones that are necessary for the expression of sexual behavior (Allrich, 1994). More long-term chronic stress also impairs reproductive function in rats (Rivier and Rivest, 1991). Lameness in dairy cows could be used as a model for a biologically occurring chronic stressor as

it is a painful long-term stressful condition associated with poor reproductive fitness (Collick et al., 1989; Hernandez et al., 2005; Lucey et al., 1986; Melendez et al., 2003; Whay et al., 1997). Indeed, lame cows require more inseminations per pregnancy and have a lower pregnancy rate to first insemination (Collick et al., 1989). Based on progesterone concentrations measured at least twice per week, lame cows also take longer to have an ovulatory estrus and commence postpartum ovarian cyclicity (Garbarino et al., 2004; Petersson et al., 2006). It is often assumed that the physical handicap of lameness results in less intense behavioral estrus in dairy cattle; however, we maintain that the chronic stress associated with lameness impairs proper endocrine function having detrimental effects on ovarian cyclicity, estrus expression and ultimately reproductive efficiency as overt estrus expression is required for appropriately timed artificial insemination.

Estrus in cattle includes the expression of behaviors such as sniffing the vulva of fellow herd-mates, flehmen, chin resting, increased restlessness and isosexual mounting behaviors (Van Vliet and Van Eerdenburg, 1996). The intensity or frequency at

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which these behaviors are expressed may be related to ovarian hormone concentrations. Frequent estradiol measurements alongside intense monitoring of behavioral signs in dairy cows suggest a positive dose-dependant relationship between the intensity of estrus and maximum estradiol concentrations (Lyimo et al., 2000; Roelofs et al., 2004). In addition, progesterone exposure prior to estrus enhances the influence of estradiol on sexual behavior in ovariectomized cows (Carrick and Shelton, 1969; Vailes et al., 1992). Furthermore, in the ewe, prior progesterone priming is not only essential for the display of estrus but increases the intensity of estrus expression (Fabre-Nys and Martin, 1991a,b; Karsch et al., 1980). Therefore, although the mechanical limitations of lameness could result in poor physical activity during estrus in dairy cows, the chronic stress of lameness may have a fundamental impact on ovarian function, compromising the normal hormonal milieu to alter the intensity of estrous behavior. Many chronically stressful periparturient diseases are associated with poor fertility (Borsberry and Dobson, 1989; Fourichon et al., 1999). However, to date, there are no data available to quantify the impact that a chronic biological stressor, like lameness, has on hormone concentrations and subsequent intensity of estrus expression. The collection of blood samples by venepuncture can itself evoke stress responses in cattle, thus in the present study, steroid hormones were measured in milk samples to reflect changes in the peripheral circulation (Dobson et al., 1975; Dobson et al., 1986).

We hypothesize that (a) the stress of lameness disrupts progesterone, estradiol and cortisol profiles in milk surrounding the first observed estrus following the resumption of ovarian cyclicity after calving; and (b) there is an association between severity of lameness and the incidence/intensity of behavioral estrus in postpartum dairy cows.

## Materials and methods

### Experimental design, animals, feeding and housing

The study was conducted on postpartum Holstein–Friesian cows ( $n=44$ ) on a UK commercial dairy farm comprising a total of ~200 year-round calving cows. The average rolling milk yield per cow in the herd was 8300 l/year. In the winter months (February to April 2004), animals were housed in a cubicle shed with concrete flooring and were at pasture during the summer (April to November 2004). The temperatures during the study period ranged between 0.2 and 27 °C. Milking of all cows took place twice a day starting at 6:30 am and 4:30 pm. All year round, animals had access to a total mixed ration at a feed-fence after milking. Pastures were of seasonal ryegrass, Italian ryegrass and white clover. Cows randomly entered the study on Day 20 postpartum with only 12 cows included at any one time to enable detailed observation. The cows ranged between 3 and 10 years of age. Prior clinical treatments for lameness were recorded and continued following normal farm practice, e.g., regular foot-trimming, skin/hoof dusting with

antibiotic powder. Animals were presented on-farm at weekly routine fertility visits if the farmer considered they were due to be inseminated but had not been seen in estrus. These prior fertility treatments comprised either 1) a single 500 µg injection of a prostaglandin F2α (PG) analogue (cloprostenol, 2 ml, Estrumate® Schering-Plough Animal Health, Uxbridge, UK) or 2) an EAZI-Breed™ progesterone-impregnated CIDR® device (Animal Reproduction Technologies Ltd., Leominster, UK) inserted intra-vaginally for 8 days with or without a single 500 µg cloprostenol injection 7 days later. These treatments were administered as deemed clinically appropriate by the attending vet who did not know the details of the study. Additionally, the farmer was not aware of on-going results during the study. Individual cows were monitored from Day 20 postpartum until the first observed estrus after the commencement of postpartum ovarian cyclicity (based on milk progesterone metabolite concentrations).

### Lameness scoring

Individuals were scored for lameness (score 1–3) one afternoon every 2 weeks from approximately Day 20 postpartum onwards, based on gait and posture while walking and standing using methods adapted from Sprecher et al. (1997) and defined in Table 1. Each individual was scored on average  $4.5 \pm 0.3$  times during the study. An individual's mean lameness score throughout the study and the lameness score taken just prior to estrus was the same in 97.8% cases. Additionally, 91% of individuals had the same or one lameness score that was  $\pm 1$  for the duration of the study. Therefore, individuals were retrospectively grouped based on their mean lameness score [nonlame (1.0–1.5)  $n=18$ ; moderately lame (1.6–2.5)  $n=9$ ; severely lame (2.6–3.0)  $n=9$ ].

### Hormone assays

Daily milk samples were taken immediately prior to afternoon milking to determine concentrations of progesterone metabolites, estradiol and cortisol. Milk progesterone metabolites [hereafter referred to as progesterone as this is the predominant progestagen in bovine milk (Purdy et al., 1980)] and cortisol samples were analyzed every other day from Day 20 postpartum to ~10 days following observed estrus. Daily samples for estradiol measurements were then analyzed around periods of potential estrus (indicated by low progesterone concentrations).

### Enzyme immunoassays

Milk progesterone and cortisol were analyzed by previously described enzyme immunoassays (EIA; Young et al., 2004 adapted from Munro and Stabenfeldt, 1984). Each EIA utilized an antibody (monoclonal antiserum progesterone metabolite Quidel Clone #425; or, polyclonal cortisol antiserum R4866; supplied by CJ Munro, University of California, Davis, CA), horseradish peroxidase conjugated label [progesterone or cortisol; prepared according to Munro and Stabenfeldt (1984)] and standards (progesterone or cortisol; Sigma-Aldrich, UK). The modified assay procedures were as follows: i) antiserum was diluted at 1:10,000 for progesterone, and 1:8500 for cortisol; ii) standards (progesterone, 4–200 pg/well or cortisol, 3.9–1000 pg/well) or undiluted samples were loaded (50 µl/well for both assays) onto each plate; and iii) the horseradish peroxidase conjugate was used at a dilution of 1:33,000 for progesterone or 1:40,000 for cortisol. The progesterone antiserum cross-reacted with several progesterone metabolites including: 4-pregnen-3, 20-dione (progesterone) 100%; 4-pregnen-3α-ol-20-one 188%; 4-pregnen-3β-ol-20-one 172%; 4-pregnen-11α-ol-3,20-dione 147%; 5α-Pregnan-3β-ol-20-one 94%; 5α-Pregnan-3α-ol-20-one 64%; 5α-Pregnan-3, 20-dione 55%; 5β-Pregnan-3β-ol-20-one 12.5% and  $\leq 10\%$  for all

Table 1  
Lameness scoring scale

Lameness score	Description	While standing	While walking	Gait
1	Nonlame	Level back posture	Level back posture	Normal
2	Moderately lame	Level back posture	Arched back	Normal to short-striding
3	Severely lame	Arched posture	Arched back	Takes one step at a time; reluctant to bear weight on one or more limbs/feet

The above scale is based on a previously described 5-point scale (Sprecher et al., 1997) where the above scores of Scores 1, 2 and 3 are comparable to the scores of 1, 2 and 3–5 on the Sprecher 5-point scale, respectively.

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