Administration of ketoprofen affects postpartum lying behaviours of Holstein dairy cows regardless of whether parturition is assisted

Nicola Gladden[1], Kathryn Ellis[1], Jessica Martin[2], Dorothy McKeegan[3]*

1. Scottish Centre for Production Animal Health and Food Safety, University of Glasgow, School of Veterinary Medicine, Bearsden Road, Glasgow, G61 1QH, United Kingdom
2. The Royal (Dick) School of Veterinary Studies and The Roslin Institute, Easter Bush Campus, University of Edinburgh, Midlothian, EH25 9RG, United Kingdom
3. Institute of Biodiversity, Animal Health & Comparative Medicine, University of Glasgow School of Veterinary Medicine, Bearsden Road, Glasgow, G61 1QH, United Kingdom

*Corresponding author: n.gladden.1@research.gla.ac.uk

Abstract

Background: Assistance at parturition in cattle is common. Although analgesia is commonly provided, its value in terms of welfare improvement is not known. Few previous studies include both parturition assistance and analgesic treatment status as well as appropriate control groups in a factorial design.

Methods: Seventy-two Holstein cattle (37 assisted and 35 unassisted) were randomly allocated to receive a single dose of the NSAID ketoprofen or a saline placebo within 3h of parturition. Detailed behavioural observations allowed a time budget to be constructed for each cow for 48 h postpartum.

Results: Cows experiencing assisted parturition spent more time in lateral recumbency (overall and with the head rested) ($p < 0.05$) – an infrequently adopted posture suggestive of illness or pain. Cows treated with ketoprofen spent less time in lateral recumbency ($p < 0.05$) (overall and with the head rested); this was independent of assistance status and lateral recumbency was not affected by the interaction between assistance and treatment status. Additionally, cows treated with

ketoprofen spent more time with the head rested when in sternal recumbency (p = 0.009) – a behaviour associated with comfortable resting.

Conclusion: These differences in lying postures exhibited by cows receiving ketoprofen analgesia suggest that, regardless of whether parturition is assisted, a single dose of ketoprofen in the immediate postpartum period has the potential to improve cow comfort in the first 48 h postpartum.

***Keywords**: Cow, Parturition, Dystocia, Analgesia, Welfare, Behaviour*

Introduction

Parturition is a necessary event for farmed cattle that is widely considered to be both painful and stressful, particularly when dystocia occurs.[1–5] Reports of dystocia prevalence in dairy cattle range from 2% to 7%[6] although the prevalence of assisted parturition consistently exceeds this, being reported by some authors as high as 50%.[6,7] This apparent disparity between the prevalence of dystocia and parturition assistance suggests that cattle are routinely experiencing assisted parturition in the absence of dystocia. However, despite parturition assistance being common, research into how it affects welfare is limited. Some behavioural differences suggestive of heightened pain in cattle following assisted parturition (compared to unassisted parturition) have been identified.[8,9] Barrier et al.[8] found that assisted cows spent less time self-grooming than unassisted cows in the first 3h postpartum and Proudfoot et al.[9] reported that cows experiencing dystocia had more standing bouts (indicative of restlessness) than cows experiencing normal parturition both 48 h pre-partum and 48 h postpartum. Furthermore, Houwing et al. (1990) found that assisted cows took longer to stand after parturition than unassisted cows;[10] however, as the calf was immediately moved in front of the cow in this study, it cannot be certain whether observed behavioural differences were due to pain, or as a result of this management practice.

Although a normal physiological response, pain in animals is associated with a suboptimal welfare status,[11] therefore any avoidable or prolonged pain related to the widespread provision of parturition assistance in the cattle industry has the potential to adversely affect the welfare of a large number of animals. It has been suggested that assisted parturition may be more painful than unassisted parturition - even if performed in the absence of dystocia - due to an increased likelihood of iatrogenic soft tissue trauma,[8,12] although data to support this notion are absent. The use of non-steroidal anti-inflammatory drugs (NSAIDs) in cattle following surgical procedures has been consistently shown to reduce post-operative pain[13] but fewer studies report the effect of NSAIDs on postpartum pain, even though they are commonly used by veterinary surgeons following dystocia.[4] Moreover, most studies report the effects of blanket treatment of all cows rather than targeted treatment of cows experiencing assisted parturition[14–16] whereas others only report effects in cows experiencing assistance and do not include unassisted controls.[17,18] Additionally, the effects of postpartum analgesia on production parameters such as milk yield and reproductive performance[14,15] are usually the focus of existing studies with very few including measures more typically considered to be sensitive indicators of welfare status, such as behaviour; for example, Swartz et al. (2018) reported the effects of peri-partum NSAID administration on the postpartum activity of cows but did not include detailed behavioural analysis.[19] Given that pain is a primary welfare concern, determining whether the provision of immediate postpartum analgesia provides welfare benefits to cattle experiencing appropriately assisted parturition is crucial.

The objective of this study was to investigate the behavioural effects of ketoprofen administration in the immediate postpartum period on the behaviour and welfare of cows experiencing both assisted and unassisted parturition. Previously, we demonstrated that immediate postpartum administration of ketoprofen to calves experiencing both assisted and unassisted birth improved calf welfare in the first 48 h of life[20] and we hypothesised that similar effects would be identified in their dams.

Materials and Methods

Farm management and animal husbandry

A 700 cow Holstein dairy herd in Scotland, UK was recruited to take part in the study; approximately 60 cows calve each month in a year-round pattern. Cows enter the dry period eight weeks before expected parturition and are initially housed in cubicles before being moved to a straw-bedded group pen three weeks before expected parturition. Parturition occurs in this pen after which cows are moved to an adjacent postpartum pen at, or before, the next milking after parturition. Each pen accommodates a maximum of 25 animals, allowing for 9 m^2 lying space and 90 cm of feed bunk space per cow. Transition cows (cows in the last three weeks of the dry period) are fed a straw-based total mixed ration (TMR) *ad libitum*; this diet is formulated to have a dietary cation-anion difference (DCAD) of −100 mEq/kg to −200 mEq/kg. Postpartum animals are fed a grass-silage based TMR *ad libitum* and all animals have *ad libitum* access to fresh water. Food is available to cows 24 h per day; cows do not receive any additional food in the milking parlour. Calves are separated from cows within 8 h (usually within 4 h) of parturition and newborn calf management is as reported in previous work.[20,21]

Postpartum cows are examined during the first week after parturition by a veterinary surgeon from the University of Glasgow School of Veterinary Medicine farm animal department. Animals that have disease diagnosed at the postpartum examination are treated under the veterinary surgeon's direction and re-examined at weekly intervals until they are 'signed off' by the attendant veterinary surgeon.

Study design and animal recruitment

A two by two randomised control trial was designed to include cows (and their calves, reported elsewhere[20,21]) experiencing assisted parturition, as well as time-matched controls experiencing unassisted parturition as described previously.[20,21] The requirement for assistance was determined by an experienced stockman who also graded the severity of assistance on a 1 to 4 scale as described by Gladden et al.[20,21] Briefly, score 1 represented unassisted parturition, scores 2 and 3 represented

mild and severe farmer assisted parturition respectively and score 4 represented veterinary assisted parturition (score 4 animals were not included in our studies). Cows and their calves were recruited as pairs as part of a wider study; cows were only recruited if their calf met the inclusion criteria described previously (live, female, Holstein).[20] No lame cows were recruited. Following recruitment, cows were randomly allocated to either a treatment group or a placebo group as previously described[20,21] and administered either a single dose of ketoprofen (treatment group) (Ketofen 10%, Merial Animal Health, Essex, UK) at the manufacturer's recommended dose rate (3mg/kg), or a single dose of saline (placebo group) (Vetivex No. 1, Dechra Veterinary Products, Shrewsbury, UK) at the equivalent volume dose rate (1ml/33kg). Ketoprofen was selected for use in this study as it has known analgesic efficacy in cattle, is licensed in the UK for use in the periparturient period and has a zero milk withdrawal period.[22] Both treatments were administered by deep intramuscular injection within three hours of parturition. Recruited animals were marked on the lumbo-sacral region with agricultural marker spray and photographs were taken to aid identification during video analysis. Body condition score of each cow was measured on a five point scale (0.25 point graduations) using the Penn-State method[23] at parturition and at 60 d postpartum as part of the wider study. Records for all cows regarding treatment type and dose administered were kept on farm and were not available to the observer during behavioural analysis.

Recruitment occurred during a single ten month period between March and December 2016. Sample size was based on power calculations performed as part of the wider study.[21] In total, 94 cows were recruited to the study by the end of the data collection period; however, due to technical failure, substantial video footage (defined as ≥ 24 h) for some animals was lost and these were not included in behavioural analysis. Seventy-two cows were included in the final behavioural analysis; 37 cows experiencing assistance at parturition (17 treatment and 20 placebo) and 35 cows not experiencing assistance at parturition (18 treatment and 17 placebo).

Sequential coccygeal blood samples were taken from all recruited cows on the day of parturition and also 24 h, 48 h and 7 d after parturition for biochemical analysis as part of a wider study reported elsewhere.[21] All cows were inspected at both the time of recruitment and the end of their data collection period by a veterinary surgeon; cows showing any signs of clinical illness at the time of parturition were not recruited. The study was performed under UK Home Office Project and Personal Licence authority. All animals were discharged from the controls of the Animals (Scientific Procedures) Act after the experiment and rehomed to the herd.

Data collection and behavioural monitoring

Closed circuit television (CCTV) cameras (Sony CCD, Vari-focal, 700 TV L, Sony, Tokyo, Japan) were set up to continuously film the parturition and postpartum pens. Footage was continuously filmed and stored on digital video recorders (DVR) (Guardian II+DVR 8 Channel, Digital Direct Security, Huntingdon, Cambridgeshire, UK) on farm.

Dam parity data were obtained from farm records and calf birthweight data were obtained during collection of calf data as part of the wider study.[20,21] A diagnosis of postpartum disease was recorded if retained foetal membranes, metritis, subclinical ketosis, left displaced abomasum (or a combination) was diagnosed by a veterinary surgeon in the first eight days postpartum. Data regarding postpartum disease diagnoses were obtained from both veterinary and farm records.

Behavioural analysis

An ethogram developed in a pilot study (McGann et al., unpublished) [Table 1] was used in data analysis. Behavioural observations began at the point that parturition was complete: this was defined as the point at which the whole body of the calf was expelled. Behavioural data were collected using instantaneous sampling[24] and a 48 h time budget was constructed for every cow. Following initial work, it was established that a sampling interval of every 20 min, every alternate hour was appropriate. Every even hour of the first 48 h postpartum was analysed (i.e. 0 h, 2 h, 4 h

and so on up to, and including, 48 h postpartum). To allow for the effect of time (relative to parturition) to be studied, behavioural observations were considered in four 12 h time blocks; 0 to 12 h, 12 to 24 h, 24 to 36 h (18 observations each) and 36 to 48 h (21 observations as the 48th hour was also included). Thus, a total of 75 observations were recorded for each cow. All behaviours were recorded in a spreadsheet (Microsoft Excel 2013, Redmond, WA, USA) in the form of letter codes corresponding to the ethogram [Table 1]. 'Primary' behaviours (lying behaviours, standing and walking) were recorded in all time points that the cow was visible whereas 'secondary' behaviours (social behaviours, grooming behaviours, feeding and drinking directed behaviours) were exhibited concurrently with primary behaviours and were recorded only when observed.

During analysis of video footage, the observer was blind to the treatment status of the animal. Due to the point at which observations started, it was not possible for the observer to be blinded to the assistance status of subjects.

Statistical analysis

All data were summarised in a spreadsheet (Microsoft Excel 2013, USA) and each behavioural observation was counted for every cow. Both a total time budget (number of observations for each behaviour divided by the total number of time points) and a visible time budget (number of observations for each behaviour divided by the number of time points the subject was visible) were produced; for the purposes of analysis, the visible time budget was used. Data were exported to Minitab (Minitab v.18, Minitab LLC., State College, PA, USA) for activity budget calculations and to Genstat (Genstat v.19, VSN International, Hemel Hempstead, UK) for inferential statistical analysis. Behavioural comparisons were conducted via Generalised Linear Mixed Models (GLMM) (Poisson distribution or negative binomial) dependent on over dispersion, using F statistics and $p < 0.05$ threshold level to define statistical significance. All models had the dispersion parameter fixed to one and all models included cow identity (ID) as a random effect. All fixed effects were treated as factors and all interactions between factors were included in maximal models. All minimal models

included treatment (i.e. ketoprofen or placebo treatment), assisted/unassisted parturition, time period, parity (multiparous/primiparous) and postpartum disease (yes/no). The interactions between treatment and assisted/unassisted parturition; treatment and time period; and assisted/unassisted parturition and time period were also included in all models. Calf birthweight was included in the models as a co-variate.

Results

Descriptive statistics

Thirty-four multiparous animals (47.2%) and 38 primiparous animals (52.87%) were recruited to the study, these are similar proportions to the wider adult population of the herd. Both treatment groups included similar numbers of primiparous (saline *n* = 22; ketoprofen *n* = 16) and multiparous animals (saline *n* = 15; ketoprofen *n* = 19). Median body condition score at parturition was 3.0 (range 2.0 to 4.5). Mean gestation length was 279 d (range 258 to 290 d) and mean calf birthweight was 44.3kg (27.6kg to 62.8kg). Eight animals (11%) were diagnosed with postpartum disease by a veterinary surgeon; six of these (75%) experienced assisted parturition.

Cows spent a marginally greater proportion of their time budget engaged in active behaviours compared to lying behaviours (53.8% compared to 46.3%); the most common behaviour overall was standing (49%) [Table 2]. Further descriptive behavioural data are presented in Table 2. The least common behaviours observed were lateral recumbency with unknown head position, lying in an unknown position, grooming others, and allowing the calf to suckle; all of which represented less than 1% of the time budget and are not further presented.

Over the whole 48 h time period, the mean proportion of the time budget where a cow was not visible was 14.7% (0% to 90.5%) and this was similar for all 12 h observation periods. The main reason cows were recorded as 'not visible' was due to cows being removed from the postpartum pen three times daily to be milked, this affected 59 cows (82%). Due to the differences in timing of

parturition relative to the (fixed) timings of daily milking, the proportion of the time budget in each 12 h block spent milking varied between cows and ranged from 0% to 33.3%. The mean proportion of time cows spent being milked in any of the four 12 h time periods was 7.8%.

Effects of assistance status

Cows that experienced assistance at parturition spent more time in lateral recumbency with their head in a rested position ($p = 0.049$) and more time in lateral recumbency in general ($p = 0.008$) than cows that did not experience assisted parturition [Table 3]. Additionally, cows that experienced assistance at parturition showed a tendency to spend more time with their head in a rested position when recumbent, irrespective of body position ($p = 0.059$).

Effects of treatment status

Cows in the NSAID treatment group spent less time in lateral recumbency with their head in a rested position ($p = 0.008$) and less time overall in lateral recumbency ($p = 0.031$) than cows in the placebo group [Table 4]. Additionally, when lying in sternal recumbency, cows in the NSAID group spent less time with the head held in an elevated position ($p = 0.038$) and more time with their head in a rested position ($p = 0.009$) than cows in the placebo group [Table 4].

Effects of interaction between assistance and treatment

Cows in the NSAID group that experienced assistance at parturition showed a tendency to spend more time engaged in feeding directed behaviours than cows in the other three interaction groups ($p = 0.079$). The interaction between assistance and treatment status did not affect any other behaviours or postures.

Effects of time period

Irrespective of treatment or assistance status, cows spent the least amount of time lying in any position in the first 12 h postpartum ($p < 0.001$) and the most time engaged in active behaviours

during the same time period ($p < 0.001$). Similarly, cows engaged in secondary behaviours for the greatest proportion of the time budget in the first 12 h postpartum ($p < 0.001$).

Cows less frequently engaged in lying in sternal recumbency with both the head elevated or rested in the 0 to 12 h and 24 to 36 h time periods than in the 12 to 24 h and 36 to 48 h time periods (both postures $p < 0.001$) [Figure 1]. This was reflected in the pattern of overall time spent lying in sternal recumbency (all head positions) and also the pattern of time spent with the head elevated (in any lying position) which were both lower in the 0 to 12 h and 24 to 36 h time periods (both postures $p < 0.001$). Lateral recumbency was not affected by time.

Effect of time period interactions

In the first 24 h postpartum there was a tendency for cows in the unassisted group to engage in drinking directed behaviours more than cows that experienced assisted parturition ($p = 0.063$). There was no effect of the interaction between treatment and time period on any behaviour or posture.

Effect of other factors

Cows that were subsequently diagnosed with postpartum disease spent more time lying in sternal recumbency with the head elevated and more time lying in sternal recumbency overall than cows that were not diagnosed with postpartum disease ($p = 0.023$ and $p = 0.013$ respectively).

Time spent engaged in lateral recumbency with the head rested was 2.5 times higher in primiparous animals than multiparous animals ($p = 0.002$) [Figure 2] and as a result, the proportion of the time budget engaged in lateral recumbency overall was also higher in primiparous animals ($p = 0.004$).

Discussion

Despite assisted parturition being a common event on farms[25] (with a prevalence exceeding that of dystocia[6]), the welfare implications of assisted parturition have rarely been studied. Assisted

parturition is thought to be more painful for cattle than unassisted parturition; however, the evidence to support this notion is lacking and there are few data assessing the effects of targeted analgesic interventions in postpartum cattle. The 2 x 2 factorial design of this study allowed us to investigate the effects of both assistance at parturition and analgesia administration as well as their interaction using detailed behavioural analysis – a novel approach. Our findings reveal that lying behaviours are affected by both assisted parturition and ketoprofen analgesia and that lying behaviours differ at different times postpartum.

Cows in this study spent 46% of the time budget engaged in lying behaviours which is similar to the findings of previous studies.[26,27] Sternal recumbency with the head elevated was the most common lying posture adopted by cattle in this study, followed by sternal recumbency with the head rested. This is consistent with Endres *et al.* (2007) who also found that lying cows most commonly adopted an elevated head position.[28] In this study standing was the most commonly observed active behaviour, and the most frequently observed behaviour overall, accounting for 49% of the time budget. This is in contrast to previous studies that have typically found that housed dairy cattle spend more than 50% of the day engaged in standing behaviours.[28,29] In this study, cows were recorded as not visible when they were milking and it is likely that this has caused the proportion of the time budget engaged in standing behaviours to be slightly underestimated. However, as the majority of cows were affected this is unlikely to have affected the overall results.

Cows experiencing assisted parturition spent more time in lateral recumbency with the head rested and more time in lateral recumbency overall than cows that experienced unassisted parturition. We also found that cows treated with ketoprofen spent less time in lateral recumbency than cows treated with saline, irrespective of assistance status. Lateral recumbency in adult cattle is infrequently observed[28,30] and is considered to be an abnormal lying posture exhibited when animals are in pain or unwell.[31–33] Ketoprofen would be expected to reduce pain-related behaviour; therefore, our results suggest that whilst assisted parturition may be more painful than normal

(unassisted) parturition, pain is experienced after parturition in all cows and postpartum administration of ketoprofen analgesia improves comfort irrespective of parturition experience. In contrast to our findings, Barrier et al.[8] did not identify an effect of assisted parturition on lying behaviours. However, Barrier et al.[8] only analysed behaviour in the first 3 h postpartum whereas we analysed behaviour for 48 h postpartum and identified that in the first 12 h postpartum cows spend the least amount of their time budget engaged in lying behaviours. Previous studies have also found that cows engage in lying behaviours less during the first 24 h postpartum than later on[34,35] and one study found that cows spent the least amount of time lying in the first 6 h postpartum.[36] It is therefore possible that the immediate postpartum behavioural analysis performed by Barrier et al.[8] missed differences in lying behaviours identified using a longer observation period. It is also possible that the degree of pain experienced several hours after postpartum may be greater than pain experienced in the immediate postpartum period. This phenomenon has been reported by multiparous women,[37] however it is unknown if this also occurs in non-human animals. Swartz et al.[19] found that cows experiencing dystocia that were treated with meloxicam were less active than control cows (as measured by step count) and overall, dystocic cows showed fewer lying bouts of longer duration than eutocic cows. Whilst in our study, the total proportion of the time budget engaged in lying or active behaviours was not affected by treatment or assistance status, we did identify effects of both assistance and treatment status on lying postures. Differences in data collection methods may explain the differences in findings; Swartz et al.[19] monitored activity using accelerometers which allowed continuous monitoring of activity, but did not allow for specific lying postures to be identified. We also found that, when lying in sternal recumbency, cows treated with ketoprofen spent more time with the head rested than cows treated with saline (irrespective of assistance status). Cows typically lie in sternal recumbency with their head resting on the body or on the ground when in deep sleep (i.e. rapid eye movement sleep).[38] Stress has been shown to reduce the duration of sleep in cattle[38] and it is possible that pain has a similar effect. It needs to be considered however that the stress of separation from the calf may also have affected sleep and

activity patterns in the cattle on this study. As all cows were separated from their calves at similar times (relative to parturition) this will have affected all animals similarly, but we were unable to control for individual differences in responses to cow-calf separation that may be determined by each individual's temperament. Nevertheless, due the randomised design of this study, animals with a temperament predisposed to experiencing a greater stress response were no more or less likely to be allocated to the ketoprofen treatment group.

In this study we found that cows engaged in sternal recumbency (both head postures and overall) more in the 12 to 24 h and 36 to 48 h time periods than in the 0 to 12 h and 24 to 36 h time periods. Lying in sternal recumbency is a normal resting behaviour for cattle[39] and is also a posture adopted when cattle are chewing the cud. Postpartum cattle have been shown to rest more at night than during the day[27] but as time in this study was relative to the (variable) timing of parturition and did not indicate time of day, it is unlikely that this observed pattern of resting behaviour is related to circadian rhythms. Rather, this finding may reflect effects of parturition on daily resting patterns of cattle that last for up to 48 h and may possibly represent a normal recovery period following parturition where resting patterns are altered compared to later in lactation. To our knowledge this is the first study to report detailed postpartum behavioural observations in 12 h intervals and more study is warranted to investigate this finding further.

Non-steroidal anti-inflammatory drugs are commonly used in bovine medicine[33] and a number of different NSAIDs are licensed in the UK for use in cattle, including ketoprofen.[22] Ketoprofen is widely used in cattle, is well tolerated,[40] and has known efficacy. In the UK and Europe, milk from animals treated with ketoprofen does not need to be discarded and ketoprofen is licensed for use in the periparturient period,[22] making ketoprofen an appropriate choice of NSAID for use in the immediate postpartum period. Ketoprofen has been shown to inhibit prostaglandin E_2 and β-glucuronidase for 24 h following administration in calves[41] and is thought to have a duration of action of approximately 24 h in cattle. In this study, cows treated with ketoprofen showed behavioural differences indicative

of increased comfort across the whole 48 h time budget and there was no interaction between ketoprofen treatment and time period. This finding may indicate that in the context of postpartum pain, ketoprofen may provide analgesia for longer than the expected 24 h. Although the pharmacokinetics of ketoprofen indicate that the expected duration of analgesic effect of ketoprofen is 24 h,[41] related work in calves has also found that ketoprofen clinically improved comfort for the first 48 h of life[20] and a prolonged duration of analgesic effect has also been reported when ketoprofen has been used in lameness studies.[42] Whilst this has been postulated to be due to reduced clearance from inflamed tissues[43] the reason for this apparent disparity between the pharmacokinetics of ketoprofen and the clinical response to ketoprofen is currently uncertain and deserves further study.

Primiparous animals, irrespective of treatment or assistance status, spent a greater proportion of the total 48 h time budget lying in lateral recumbency with the head rested than multiparous animals. This suggests that primiparous cows may experience more pain following parturition (irrespective of assistance status) than multiparous cows and that this pain may be less responsive to NSAID analgesia. It is possible that due to their smaller size and lack of parturition experience, more tissue trauma occurs during parturition in primiparous cows, although plasma creatine kinase concentration (a biomarker of muscle damage) following parturition in the same animals as those reported here was not affected by age.[21] If primiparous cows do experience more pain in the immediate postpartum period than multiparous cows, this would be an interesting contrast to some human and pig studies that have reported increased postpartum pain in multiparous patients.[37,44] Although a parity effect was identified in this study, the proportion of the time budget engaged in lying in lateral recumbency with the head rested was small for both multiparous and primiparous animals and as such, parity differences in pain experienced by cows both at parturition and in the postpartum period requires further study. Such data could allow farmers to optimise the parturition management of these groups and further improve bovine welfare in the periparturient period.

Drinking directed behaviours tended to be affected by the interaction between assistance status and time period, with cows experiencing assisted parturition tending to spend less time engaging in drinking directed behaviours than unassisted controls in the first 24 h postpartum. This is in contrast to results reported by Proudfoot et al.[9] who found that drinking time did not differ between cows experiencing dystocia and cows experiencing normal parturition, although cows that had experienced dystocia tended to consume more water than cows experiencing unassisted parturition.[9] The tendency towards differences in drinking directed behaviours identified in this study may be related to heightened pain experienced by assisted cows in the first 24 h postpartum; however, although painful events have been shown to affect lying behaviours in cattle[45,46], the effect on drinking behaviours is less certain and other factors affecting behaviour, such as exhaustion, may contribute to this finding.

The factorial design used in this study is considered to be gold standard for establishing whether a behaviour is affected by pain or another factor[47] although different types of pain and variations in analgesic efficacy mean that an absence of response to analgesia does not necessarily indicate the absence of pain.[48] A further limitation of this study is the amount of time cows were not visible, which may have meant some behaviours were under-represented in the analysis. However, there were no differences between assistance or treatment groups in the proportion of the time budget the cows were not visible and a large proportion of the 'not visible' time was due to the time cows spent being milked (during which time they were not in the postpartum pen). This was unavoidable as cameras could not be positioned in the milking parlour and, as the timing of parturition could not be fixed in relation to the time of milking, it was not possible to ensure all cows were affected for an equal proportion of the 48 h time budget. Nonetheless, as the majority of cows were affected, and the primary behaviour of all cows is the same during milking (i.e. standing), it is unlikely that this had an effect on our results.

Parturition assistance on the study farm is primarily provided by one experienced stockman and early intervention with parturition assistance is only performed if the calf is malpresented. This is an approach that is considered to optimise outcomes for both the cow and the calf;[49] however, it may mean that cows experiencing even score 3 assisted parturition in this study did not experience as much soft tissue trauma and associated pain as cows managed in a less optimal manner. Furthermore, only parturition events that produced live female Holstein calves were included in this study which may also have minimised the degree of soft tissue trauma experienced by recruited cows. It is difficult to determine whether cows experiencing less optimised parturition assistance would also experience improved comfort following postpartum ketoprofen treatment. However, pre-surgical NSAID treatment of cows experiencing caesarean section has been shown to improve postpartum comfort[17] which suggests that peripartum NSAIDs may lead to reduced pain and improved welfare in cows experiencing all types of parturition assistance.

Although individual effects of treatment status and assistance status were identified in this study, we did not identify any interactions between treatment and assistance status. Nevertheless, cows experiencing assisted parturition that received ketoprofen showed a tendency to spend a greater proportion of their time budget engaged in feeding directed behaviours than other subjects. Although instantaneous sampling intervals of 20 min have been shown to represent standing and lying behaviours well[50,51], feeding behaviours are better represented by shorter sample intervals.[51] Therefore, it is possible that the proportion of time spent feeding was underestimated in this study and increasing the frequency of sampling may have increased the reliability of this finding. The importance of maintaining feed intake in postpartum cattle cannot be overstated and the tendency for ketoprofen administration to increase the proportion of time spent at the feed face in cows experiencing assisted parturition warrants further attention.

Cows that experienced postpartum disease engaged in sternal recumbency with the head held up more than cows that did not experience postpartum disease which was unexpected. Previous

studies have found that lying times of cattle experiencing postpartum disease are increased when compared to unaffected cattle.[52,53] Whilst neither of these studies report lying postures, the differences in lying times described differ to our findings which suggested that — although posture was affected by postpartum disease — total proportion of the time budget spent lying was not affected. This may be due to differences in study design, but very few of the animals in our study experienced postpartum disease; therefore, whilst interesting, the unexpected effect of postpartum disease on lying posture identified in this study needs to be interpreted with caution.

Conclusion

The effects of ketoprofen treatment on lying postures observed in this study suggest that parturition of all types is associated with pain and all cows may benefit from postpartum analgesia, regardless of parturition experience. Cows experiencing assisted parturition engaged in abnormal lying postures for a greater proportion of their time budget than cows experiencing unassisted parturition, supporting the notion that assisted parturition may be a greater challenge to welfare. However, no assistance/treatment interaction effects on lying postures were observed which may be because all cows were experiencing pain after parturition, or because there are other factors adversely affecting the welfare of cows following assisted parturition that could not be measured (e.g. exhaustion). The results of this study indicate that a single administration of ketoprofen in the immediate postpartum period has the potential to improve the welfare of post-parturient dairy cows for at least 48 h. As such, the routine use of ketoprofen should be considered by both farmers and veterinary surgeons to optimise postpartum welfare.

Acknowledgements

Funding and product were provided by Merial Animal Health (now a part of Boehringer Ingelheim Animal Health). Additional funding was provided by the University of Glasgow James Herriot Fund and the James Houston Crawford Endowment Fund. The funders were not involved in study design,

data collection, data interpretation or publication. Ketofen 10% was manufactured and provided by Merial Animal Health at the time of the study; this product is now owned and produced by CEVA Animal Health Ltd. (Amersham, UK). The authors would like to thank Allison McGann, all of the farm staff and University of Glasgow School of Veterinary Medicine BVMS final year students for their invaluable assistance.

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Table 1: Ethogram used to describe cow behaviours. See electronic supplementary material for examples of lying postures.

Category	Behaviour/posture	Description	Code
Lying behaviours	**Lateral**	Cow is lying on one side with both forelimbs on one side of the body. The shoulder is in contact with the ground. This is subdivided into 'head up' [U] (head is held in an elevated position not in contact with the ground or any part of the body) or 'head down' [H] (head is held in a rested position and any part of the head or face has contact with either the ground or any part of the body). If the body position can be identified but the head position cannot, head position is recorded as 'unknown' [K].	R [U/H/K]
	Sternal	Cow is lying in sternal recumbency with the ventral sternum in contact with the ground; the shoulder does not contact the ground. Limbs may be positioned either one on each side of the body or both on the same side of the body. This posture is also subdivided into 'head up' [U] (head is held in an elevated position not in contact with the ground or any part of the body) or 'head down' [H] (head is held in a rested position and any part of the head or face has contact with either the ground or any part of the body). If the body position can be identified but the head position cannot, head position is recorded as 'unknown' [K].	N [U/H/K]
	Unknown	Cow is known to be lying but the posture cannot be identified. If the head position also cannot be identified, head position is recorded as 'unknown' [K]. If the head position can be identified, this is recorded as either 'head up' [U] (head is held in an elevated position not in contact with the ground or any part of the body) or 'head down' [H] (head is held in a rested position and any part of the head or face has contact with either the ground or any part of the body).	K [U/H/K]
Active behaviours	**Standing**	Cow is standing still. All four feet are in contact with the ground, the body is not in contact with the ground.	T
	Walking	The cow takes two or more consecutive steps in a forward direction.	W
Secondary behaviours	**Feeding directed**	The cow is positioned at the feed face with the head positioned between the bars of the head yokes to enable access to feed	F
	Drinking directed	The cow is positioned at the water trough with the face/nose directed to the water	D
	Self-grooming	Cow is licking/nibbling/scratching self	S
	Grooming others	Cow is licking/nibbling/scratching other adult cow(s)	O
	Social behaviours	Non-grooming behaviours directed to other adult cows in the group. Includes chin-resting, head rubbing, suckling and mounting other adult cows.	B
	Investigating calf	Cow engages in licking/nuzzling/sniffing/head rubbing the calf	C
	Allowing suckling	Cow allows calf to teat seek and/or suckle. Calf is seen with nose intentionally at the ventral abdomen and/or udder or teats and this behaviour is allowed and/or encouraged by the cow	A
Other behaviour		Cow engages in behaviours or postures not described elsewhere in the ethogram. This includes interaction with humans.	X
Not visible		Cow is not visible or not identifiable on camera footage for the sample point scored	V

Table 2: Overall summary statistics (mean, standard error [± SE] and range) of raw time budget data for all animals combined into a single group (*n* = 72); data are presented as a proportion of time budget (%). Overall proportion of time engaged in lateral recumbency with unknown head position, lying in an unknown position, grooming others, and allowing the calf to suckle was less than 1% for each behaviour and data are not presented

Behaviour	**Proportion of time budget (%)**			
	Mean	**SE**	**Minimum**	**Maximum**
Total combined active behaviours	53.8	1.09	7.14	100.0
Total combined lying behaviours	46.3	1.09	0.00	92.9
Standing	49.0	1.06	5.88	100.0
Walking	4.76	0.37	0.00	31.3
Lying sternal, head up	26.2	0.94	0.00	83.3
Lying sternal, head down	9.73	0.55	0.00	41.2
Lying sternal, head position unknown	3.85	0.42	0.00	45.5
Lying lateral, head up	1.25	0.19	0.00	14.3
Lying lateral, head down	2.15	0.28	0.00	27.8
Lying posture and head position unknown	2.61	0.32	0.00	29.4
Feeding directed behaviours	15.2	0.66	0.00	55.6
Drinking directed behaviours	1.70	0.21	0.00	16.7
Grooming self	1.75	0.21	0.00	26.7
Investigating calf	2.77	0.35	0.00	38.5
Other behaviours	1.21	0.20	0.00	20.0
Not visible	14.7	0.82	0.00	90.5

Table 3: Back-transformed mean and standard error (SE) and statistical differences (*p*-value and F-statistic) of proportion of visible time budget (%) engaged in different behaviours for cows experiencing assisted and unassisted parturition, irrespective of treatment status. Bold font indicates statistical significance ($p < 0.05$).

Behaviour	Assisted parturition			Unassisted parturition			F statistic	*p*-value
	Mean (± SE)	Min (%)	Max (%)	Mean (± SE)	Min (%)	Max (%)		
Lying in sternal recumbency, head elevated	26.9 ± 1.06	0.00	64.3	29.6 ± 1.07	0.00	83.3	0.77	0.381
Lying in sternal recumbency, head rested	11.0 ± 1.09	0.00	41.2	10.3 ± 1.12	0.00	38.9	1.73	0.190
Lying in lateral recumbency, head elevated	1.13 ± 1.37	0.00	14.3	0.08 ± 584	0.00	14.3	0.90	0.343
Lying in lateral recumbency, head rested	1.28 ± 1.41	0.00	27.8	1.02 ± 1.45	0.00	15.4	**3.92**	**0.049**
Walking	3.63 ± 1.20	0.00	31.3	3.06 ± 1.22	0.00	20.0	2.19	0.140
Standing	45.5 ± 1.23	5.90	100	46.4 ± 1.26	8.30	100	0.01	0.907
Feeding directed behaviour	15.7 ± 1.09	0.00	55.6	14.2 ± 1.06	0.00	50.0	0.54	0.465
Drinking directed behaviour	1.04 ± 1.35	0.00	16.7	1.72 ± 1.78	0.00	16.7	0.40	0.526
Grooming self	1.59 ± 1.26	0.00	12.5	1.95 ± 1.29	0.00	26.7	1.15	0.285
Total lying in sternal recumbency (any head position)	42.5 ± 1.04	0.00	92.9	43.4 ± 1.05	0.00	83.3	0.04	0.843
Total lying in lateral recumbency (any head position)	2.78 ± 1.27	0.00	37.5	1.87 ± 1.31	0.00	28.6	**7.12**	**0.008**
Total head elevated (all body positions)	28.4 ± 1.06	0.00	64.3	30.6 ± 1.09	0.00	83.3	0.29	0.588
Total lying	48.6 ± 1.04	0.00	92.9	47.6 ± 1.03	0.00	91.7	0.36	0.550
Total active behaviours	50.1 ± 1.04	7.10	100	50.3 ± 1.04	8.30	100	0.42	0.519
Total secondary behaviours	22.8 ± 1.06	0.00	66.7	22.0 ± 1.08	0.00	69.2	0.13	0.720

Table 4: Back-transformed mean and standard error (SE) and statistical differences (*p*-value and F-statistic) of proportion of visible time budget (%) engaged in different behaviours for cows treated with ketoprofen or saline placebo, irrespective of assistance status. Bold font indicates statistical significance ($p < 0.05$).

Behaviour	Ketoprofen			Placebo			F statistic	*p*-value
	Mean (± SE)	Min (%)	Max (%)	Mean (± SE)	Min (%)	Max (%)		
Lying in sternal recumbency, head elevated	25.8 ± 1.06	0.00	65.0	30.7 ± 1.07	0.00	83.30	**4.35**	**0.038**
Lying in sternal recumbency, head rested	12.1 ± 1.10	0.00	41.2	9.33 ± 1.15	0.00	33.30	**7.02**	**0.009**
Lying in lateral recumbency, head elevated	0.34 ± 24.5	0.00	14.3	0.27 ± 24.5	0.00	14.30	0.35	0.554
Lying in lateral recumbency, head rested	0.77 ± 1.45	0.00	18.2	1.70 ± 1.40	0.00	27.80	**7.24**	**0.008**
Walking	3.79 ± 1.19	0.00	25.0	2.93 ± 1.23	0.00	31.30	2.19	0.685
Standing	46.9 ± 1.23	5.90	100	45.0 ± 1.25	7.10	100	0.01	0.915
Feeding directed behaviour	14.8 ± 1.09	0.00	55.0	15.1 ± 1.10	0.00	50.00	0.54	0.465
Drinking directed behaviour	1.22 ± 1.32	0.00	16.7	1.47 ± 1.31	0.00	16.70	0.40	0.526
Grooming self	1.73 ± 1.26	0.00	13.3	1.80 ± 1.28	0.00	26.70	0.10	0.756
Total lying in sternal recumbency (any head position)	42.6 ± 1.04	0.00	83.3	43.4 ± 1.05	0.00	92.90	0.02	0.894
Total lying in lateral recumbency (any head position)	1.83 ± 1.29	0.00	18.2	2.85 ± 1.28	0.00	37.50	**4.69**	**0.031**
Total head elevated (all body positions)	27.1 ± 1.06	0.00	65.0	32.0 ± 1.07	0.00	83.30	**4.14**	**0.043**
Total lying	47.4 ± 1.04	0.00	83.3	48.9 ± 1.04	0.00	92.90	0.21	0.648
Total active behaviours	51.2 ± 1.04	16.7	100	49.2 ± 1.04	7.10	100	0.35	0.553
Total secondary behaviours	22.4 ± 1.07	0.00	66.7	22.3 ± 1.07	0.00	69.20	0.12	0.731

Figure 1: Back-transformed mean (± SE) proportion of visible time budget spent lying in sternal recumbency with the head elevated (*A*) or the head rested (*B*) for each 12 h time period irrespective of assistance or treatment status (n = 72). Different letters indicate significant differences ($p < 0.001$)

Figure 2: Back-transformed mean (± SE) proportion of visible time budget spent in lateral recumbency with head rested for primiparous (n = 38) and multiparous animals (n = 34) irrespective of assistance or treatment status. Different letters indicate significant differences ($p = 0.002$).