Postpartum Ketoprofen Treatment Does Not Alter Stress Biomarkers in Cows and Calves Experiencing Assisted and Unassisted Parturition: A Randomised Controlled Trial

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Abstract

Dystocia is considered painful and stressful for both the dam and the calf although systematic evidence of this is limited. Few studies have investigated biochemical markers of stress and pain postpartum and whether any adverse effects are ameliorated by administration of analgesia. In this study, cow-calf pairs experiencing both mild to moderate farmer assistance and no assistance at parturition were randomly assigned to either treatment or placebo groups in a two-by-two design (animals subject to veterinary intervention were excluded). The treatments were the non-steroidal anti-inflammatory drug (NSAID) ketoprofen or saline, administered within three hours of parturition. Blood samples taken in the immediate postpartum period, and at 24h, 48h and 7days after parturition, were analysed for plasma concentrations of creatine kinase and cortisol (cows and calves) and plasma L-lactate and total protein concentration (calves). Stress biomarkers were highest in the immediate postpartum period and declined over time ($p<0.05$). Cow plasma cortisol was higher in animals experiencing assisted parturition in the immediate postpartum period ($p=0.023$); by 24h no difference was evident. Intervention with NSAID analgesia did not result in beneficial changes in stress biomarkers. Based on biomarkers alone, this suggests limited benefits of NSAID treatment in unassisted or mild to moderately-assisted parturition.

1. Introduction

Assisted parturition due to dystocia in cattle is considered by both farmers and veterinary surgeons to be a painful and stressful event.[1–3] The prevalence of assistance at parturition in dairy cows has been reported to range from 10 - 50%;[4] therefore, there is the potential for assisted parturition to have a significant welfare impact. There is however, a paucity of research in this area, in particular regarding the pain experienced by the dam during and after parturition and the welfare impact of assisted birth on the calf.[5,6]

A difficult calving has been shown to adversely affect the vigour of neonatal calves as assessed using criteria such as modified Apgar scores, behavioural analysis and clinicopathological measurements,[6–10] and similar findings have been reported in other domestic ruminants.[11,12] L-lactate is readily measured and correlates with blood pH, allowing it to be used as an indicator of metabolic acidosis in neonatal calves.[10,13,14] Assisted birth has been associated with increased L-lactate concentration in neonatal calves, which in turn is correlated with lower Apgar scores.[13,15] Reduced vigour in the first hour of life is associated with reduced colostrum intake[16] and a number of studies have demonstrated an association between inadequate passive transfer in calves and dystocia.[17–19]

Pain is difficult to assess in animals, although plasma cortisol concentrations are widely used in veterinary studies as a biochemical indicator of stress and pain.[20–23] Foetal blood cortisol concentration is well known to be elevated at the time of parturition, regardless of the circumstances of parturition and is a normal foetal response.[24,25] Calves experiencing dystocia are reported to have blood cortisol concentrations elevated to a greater degree than in normal calving, presumably as a result of the additional stress and pain.[26,27] Blood cortisol concentration in cows experiencing dystocia also increases to a greater degree than in cows not experiencing dystocia.[26,28,29] Creatine kinase (CK), whilst not an indicator of pain *per se*, is a muscle specific enzyme that is indicative of muscle damage[30] and may therefore be useful as a proxy measure of pain in this context. Plasma CK in cattle around parturition is less studied than cortisol; however, the limited data available suggest that more traumatic parturition is associated with increased measurement of CK concentration.[26,31]

A number of NSAIDs are licensed for use in cattle[32] and they are commonly used by both veterinary surgeons and farmers for their antipyretic, anti-inflammatory and analgesic properties. Ketoprofen is a propionic acid NSAID that is commercially available as a 50:50 racemic mixture of two enantiomers (R(-) and S(+)).[33,34] In calves, ketoprofen has been shown to have rapid clearance from plasma (within three hours); however, clearance from inflammatory exudate is much slower (twelve hours for complete clearance).[33] In adult cows, rapid plasma clearance following intravenous administration has also been demonstrated,[35] although clinical responses to ketoprofen have been demonstrated to be longer term,[36,37] suggesting that, similar to calves, tissue clearance occurs more slowly. In the UK, ketoprofen is licensed for treatment of a range of painful and inflammatory conditions in cattle and has a zero milk withdrawal period. Although not licensed for calves less than six weeks of age, the product used in this study (Ketofen 10%, Merial Animal Health Ltd.) has been used safely in calves as young as three days old.[38]

The objective of this study was to assess biochemical markers of pain and stress and their response to analgesic following farmer assisted parturition in both the cow and the calf. Cow-calf pairs receiving farmer assistance at parturition and time matched cow-calf pairs not receiving any assistance were assigned to either a treatment group (NSAID) or a placebo group in a two by two study design. Animals experiencing veterinary assisted parturition were excluded from the study as previous work has indicated that veterinary surgeons are already more likely than farmers to provide analgesia at parturition.[1,3,39] Therefore, as most calving assistance is provided by farmers,[40] there is the potential for analgesic administration at calving to have a wide-ranging welfare impact.

2. Materials and Methods

The study was performed under UK Home Office licence Project and Personal licence authority; animals were returned to the herd after being discharged from the controls of Animals (Scientific Procedures) Act after veterinary inspection.

2.1 Farm Management and Husbandry

A single, 700 cow Holstein dairy herd in Scotland, UK was recruited. Approximately 60 animals calve each month in a year-round calving system. Multiparous and primiparous animals within three weeks of their expected calving date are housed together in a large straw-bedded pen; animals calve in this group pen. Farm staff monitor the calving pen 24 hours a day and if an animal is observed to be experiencing difficulty or parturition is prolonged, animals are examined by one of two experienced farm staff for dystocia; the same two farm staff are the only two staff to assist parturition on the farm. Delivery of malpresented calves is assisted without delay; however, if the calf is normally presented, parturition progress is monitored and cows are given opportunity to calve unaided before assistance is provided. This approach aims to reduce damage to maternal soft tissues and postpartum complications that may occur in cases of inappropriately early assistance. Following parturition, cows are moved to an adjacent straw-bedded postpartum pen at, or before, the first milking after the time of parturition. Milking occurs three times daily at eight hour intervals therefore the maximum amount of time that might elapse before an animal is moved into the postpartum pen is eight hours.

Calves are removed from the dam after birth and moved to a straw bedded group calf pen (measuring 3.1m x 2.9m); on average four to six calves are housed in each pen at any one time. Cow-calf separation always occurs within eight hours of birth and most commonly within three hours of birth. Calves are fed a minimum of 4.5 litres of colostrum within four hours of birth; initially colostrum is offered from a bottle but if calves do not suckle well or do not drink the full 4.5 litres, colostrum is fed via an oesophageal feeder to ensure adequate colostrum intake. Calves that vigorously consume the initial 4.5 litres of colostrum are offered more colostrum from a bottle; the amount of extra colostrum consumed is dependent on the appetite of the individual calf. Colostrum is harvested from cows at milking time which may not occur for as long as eight hours after birth. Therefore, to ensure colostrum intake is always within four hours of birth, calves are fed stored colostrum from a cow other than its own dam; this is always from a single animal and not pooled. Calves are weighed and have the umbilicus dipped with a 7.5% iodine solution when they are moved to the calf pens. Calves are fitted with an approved ear tag within 36 hours of birth in accordance with European legislation regarding identification of cattle (regulation [EC] 911/2004).

Subsequent to the initial colostrum feed, calves are fed 3.5 litres of powdered milk replacer mixed at a concentration of 125g powder per litre of water (Provimilk Daisy, Provimi) twice daily with a group teat feeder. If calves lack the vigour to suckle, or are pushed away from the feeder by other calves, they are fed individually with a bottle or an oesophageal feeder as required.

2.2 Study design

A two by two randomised control trial was designed to include both cows (both primiparous and multiparous animals were recruited) and calves requiring assistance at parturition and unassisted control groups. Randomisation was performed using a randomly generated Latin square[41] with odd numbers representing placebo and even numbers representing NSAID treatment. Cow-calf pairs were eligible to be included in the study if the calf born was female and purebred Holstein. Recruited animals were designated as either assisted or unassisted parturition, and the level of assistance was graded, in accordance with a 1 - 4 grading system modified from Barrier *et al*.[7,17] [*Table 1*]. Cow-calf pairs experiencing grade 1 parturition were assigned to the 'unassisted' group;

cow-calf pairs experiencing grade 2 and 3 parturition were grouped together and assigned to the 'assisted' group. As this study was designed to assess effects on farmer assistance at parturition, veterinary assistance or caesarean section (grade 4) was not included.

Power calculations (Minitab v.18.1, Minitab Incorporated) based on literature data[26] indicated that a difference in plasma cortisol concentration of 93nmol/L in cows experiencing assistance and 87nmol/L in calves experiencing assistance would be detectable at 80% power if 40 assisted cow-calf pairs were recruited. Due to the time-matched design of the study, 40 unassisted controls (cow-calf pairs) were also required.

Ketoprofen (Ketofen 10%, Merial Animal Health Ltd.) was administered by deep intramuscular injection at a dose rate of 3mg/kg bodyweight (equivalent to a volume of 1ml/33kg bodyweight) to each cow-calf pair assigned to the treatment group. Saline (Vetivex 1, Dechra Veterinary Products) was administered by deep intramuscular injection at the same volume dose rate (1ml/33kg bodyweight) to each cow-calf pair assigned to the placebo group. Ketoprofen or saline was administered within three hours of parturition by either the author (NG) or the head stockman; the administrator was not blinded to the assistance or treatment status of the animal and neither administrator was involved in biochemical analysis. Which product the animal received, and the volume administered, was recorded by the administrator in a separate chart kept in the farm office for the duration of the study. Animal recruitment and data collection took place over a single period between the 7th of March and the 17th of December 2016

All cows and calves recruited to the study were marked on the lumbosacral region with coloured agricultural marker spray to identify them as being subject to UK Home Office regulations; this type of identification is not used on the farm for any other reason.

Animal identification, date and time of calving, degree of assistance provided, time of calf movement into a calf pen, pen number, calf birthweight and the volume of colostrum ingested by the calf were all recorded by farm staff. Dam lactation was obtained from farm computer records (Dairycomp 305, Valley Agricultural Software).

All cows and calves were continuously monitored using closed circuit television (CCTV) cameras (Sony CCD Vari-focal 700TVL, Sony) for 48 hours postpartum as part of a wider behavioural study.

2.3 *Blood sampling and biochemical analysis*

Jugular blood samples were obtained from calves and coccygeal blood samples were obtained from cows within six hours of parturition (immediate postpartum sample) and 24 (± 12) hours, 48 (± 12) hours and seven days after parturition for biochemical analysis. Lithium heparin anti-coagulated blood samples were obtained from each cow for cortisol and CK analysis and lithium heparin and fluoride oxalate anti-coagulated blood samples were obtained from each calf for cortisol, CK, L-lactate and total protein analysis using 20G needles and blood collection tubes (BD vacutainer, Becton, Dickinson and Company).

Samples were centrifuged for 5 minutes at 4000 x *g* (VWR Collection CompactStar CS 4 laboratory centrifuge, VWR International Ltd.). Plasma was drawn off and separated into three aliquots (one of approximately 0.2mL and two of 2 mL) for storage at -20^{o}C on farm (later moved to -80^{o}C storage).

The 0.2mL aliquot of plasma was refrigerated on farm immediately after separation for analysis of total protein.

Calf total plasma protein concentration was measured by the author using an optical refractometer (DIGIT 012 clinical pocket refractometer, CETI). All other samples were referred to Veterinary Diagnostic Services, University of Glasgow School of Veterinary Medicine, for analysis. Blood samples were analysed in batches of 20 - 30 samples, cow and calf samples were analysed separately. All analyses were performed according to International Federation of Clinical Chemistry (IFCC) parameters. Plasma CK concentration and plasma L-lactate concentration were measured using a Siemens Dimension Expand clinical chemistry analyser and the Siemens Immulite 200Xpi chemiluminesence system (Siemens Healthcare Ltd.). Plasma cortisol concentration was measured using solid phase competitive chemiluminesence on the Siemens Immulite Xpi analyser. Technicians performing biochemical analysis were blinded to both assistance and treatment status.

2.6 Statistical analysis

Raw data were entered into a spreadsheet (Excel 2013, Microsoft) and exported into statistical software (Minitab v.18.1, Minitab Incorporated) for analysis. Chi-squared analysis was performed on calving data (cow parity [primiparous vs. multiparous], time of day and calf birthweight) to test for association with assistance status. For the purposes of chi-squared analysis, calf birthweight was categorised as heavy (more than 10% above the mean), average and small (more than 10% below the mean). Time of day calved was categorised in four hour blocks as early morning (02:00 - 05:59), morning (06:00 - 09:59), midday (10:00 – 13:59), afternoon (14:00 – 17:59), evening (18:00 – 21:59) and night (22:00 – 01:59).

Normality analysis was performed on all continuous data; all data except for CK concentration (both cow and calf) were transformed using a log10 transformation.[42,43] Calf CK concentration data were transformed in Minitab using Johnson transformation[43] because data could not be adequately transformed using a logarithmic transformation. All transformed data were entered into a mixed effects model (a separate model for each analyte); animal identification was entered as a random effect. Assistance status, treatment status, time of sample (0h, 24h, 48h and 7d), interaction between assistance and treatment status, interaction between assistance status and time, interaction between treatment status and time and interaction between assistance status, treatment status and time were all entered as fixed effects. Cow lactation was entered as a covariate factor for analysis of cow data. Dam lactation and calf birthweight were entered as covariate factors for analysis of calf data. The amount of colostrum ingested was also included as an additional covariate factor for analysis of calf total protein concentration. A step backwards analysis was performed, removing factors sequentially if $p>0.1$. Model summary (S-value and R-squared) and residual plots were checked for all models to assess goodness of fit. Transformed data were back-transformed after completion of analysis; back-transformed means and confidence intervals are reported unless otherwise stated.

Cow plasma CK concentration data could not be adequately transformed; chi-squared tests for association were performed on these data to examine the association between plasma CK concentration and group (assisted treatment [AT], assisted placebo [AP], unassisted treatment [UT] and unassisted placebo [UP]) at each sampling point. For the purposes of chi-squared analysis, plasma CK concentration was categorised as either above or below the upper limit of the laboratory reference interval (196 U/L).

Spearman's rank coefficient for correlation was calculated for any significant covariate factors in the mixed effects models; correlation was only significant for L-lactate analysis. Significance was defined as $p<0.05$ for all statistical tests.

3. Results

Ninety-four cows and 95 calves were recruited; five cows and four calves were excluded [*Table 2*]. In total, blood samples from 89 cows and 91 calves were included in the final biochemical analysis [*Table 2*]. Mean calf birthweight was 43.0kg (range 27.6kg – 62.8kg); calf birthweight did not have an effect on any biomarker. Approximately half of the dams recruited were in lactation one [*Table 3*], which is reflective of the lactation distribution of the herd. Assistance status was not affected by calf birthweight, dam parity or time of day parturition occurred.

3.1 Cortisol

Plasma cortisol concentrations in both cows and calves were highest in the immediate postpartum period ($p<0.001$). There was no effect of assistance or treatment status alone, or interaction between assistance and treatment on plasma cortisol concentration in either cows or calves. Cow lactation and calf birthweight had no effect on either cow or calf plasma cortisol concentration.

Interaction between treatment and time showed a tendency to effect in calves ($p=0.05$). Plasma cortisol concentration tended to be higher in calves treated with saline placebo in the immediate postpartum period (209nmol/L [95%CI 179 - 244] compared to 198nmol/L [95%CI 174 – 225]) but by 7d postpartum showed a tendency to be lower than that in calves treated with ketoprofen (28.3nmol/L [95%CI 22.3 – 35.8] compared to 34.3nmol/L [95%CI 28.0 – 42.0]).

Interaction between calving type and time had an effect in cows ($p=0.023$), but not in calves. Plasma cortisol concentration in cows receiving assistance at parturition was 1.5 times that of cows not receiving assistance in the first time period (70.7nmol/L [95%CI 86.0 – 58.0] compared to 48.1nmol/L [95%CI 57.7 – 40.2]) [*Figure 1*]. This was a short-term effect; assistance status did not affect cow plasma cortisol concentration at any of the other sampling time points.

3.2 Creatine kinase

There was no effect of assistance group in cows either immediately or at seven days postpartum. Cows in the AT and UT groups were more likely to have plasma CK concentration above 196 U/L than cows in the AP and UP groups at both 24h postpartum (χ^2 [3, n=85] = 30.57, $p<0.001$) and 48h postpartum (χ^2 [3, n=82] = 9.87, $p=0.02$) (differing sample sizes were due to missing values).

Plasma CK concentration in calves was highest in the immediate postpartum period ($p<0.001$) and was higher in calves born to primiparous dams than in calves born to multiparous dams ($p=0.026$) [*Figure 2*]. Treatment status, assistance status or the interaction between the two factors did not have an effect.

3.3 L-lactate

Plasma L-lactate concentration decreased as time progressed (*p*<0.001) and was negatively correlated with calf birthweight (ρ=-0.156, *p*=0.004). Dam lactation was also negatively correlated with plasma L-lactate concentration (ρ=-0.142, *p*=0.007).

Assistance status, treatment status, or the interaction between the two factors, did not have an effect.

3.4 Total protein

Calf total plasma protein concentration was lowest in the immediate postpartum time period (*p*<0.001). An effect of interaction between time and treatment status was identified (*p*=0.001); total plasma protein concentration in calves treated with ketoprofen (irrespective of assistance status), was lower than in calves treated with saline at the 48h sampling point [*Figure 3*].

Assistance status, treatment status or the interaction between the two factors did not have an effect. There was no effect of amount of colostrum ingested (*p*=0.6).

4. Discussion

The results of this study provide novel information about firstly, whether assistance has an effect on key biochemical parameters in the immediate postpartum period and secondly, whether NSAID treatment affects these biomarkers. The inclusion of cows and calves with time-matched controls, as well as the two by two design of the study, allowed us to separately examine the effects of assistance and analgesic treatment.

Similar to previous research, plasma cortisol, CK and L-lactate concentrations were all highest at parturition[10,13,26,44,45] and in cows, plasma cortisol concentration immediately following parturition was highest in animals experiencing assistance.[26,28] Few studies have measured cortisol concentration in cows in the days following parturition. A pilot study of only three animals has suggested that serum cortisol concentration remains higher in cows experiencing moderate assistance at parturition for up to three days postpartum;[46] however, in this study, differences in plasma cortisol concentration between cows experiencing assisted and unassisted parturition were only evident in the immediate postpartum period. This suggests that, whilst a difficult parturition is more stressful for cattle than a normal (unassisted) parturition, this is a short lived effect for cows experiencing mild to moderate assistance. This study did not include cows experiencing more severe assistance or caesarean section, which may have longer lasting effects. Additionally, management of cattle on the farm recruited is focused on minimising cow stress and maximising cow welfare. It is possible that in different farm management systems where postpartum cows may be exposed to more chronic stressors, longer term differences between plasma cortisol concentration in cows experiencing assistance at parturition compared to those experiencing unassisted parturition may be seen. In calves born to assisted parturition, serum cortisol concentration has previously been demonstrated to be elevated;[26,27,47] in contrast, this study found that assistance had no effect on neonatal plasma cortisol concentration. Interestingly, unlike in previous studies,[10,26,48] assistance at birth did not have an effect on any biochemical parameters in the calves studied. In this study, only mild and moderate assistance were included whereas in other studies, more severe assistance and caesarean section have been included. Homerosky *et al.* (2017) reported that severe assistance (defined as assistance using a mechanical calving aid) was associated with higher L-lactate concentration in beef calves at ten minutes postpartum but mild assistance (defined as manual assistance) did not have an effect.[10] Here, assistance scores two and three were collapsed into a single category and L-lactate concentration in calves experiencing assistance was very similar to those born to unassisted parturition. It is probable that whilst severe dystocia is an adverse event regarding the health of the calf, less severe dystocia is no more adverse than a normal birth. Whilst malpresentations cannot be avoided, inappropriate intervention at parturition may result in more severe assistance being provided than might otherwise have been necessary.[40] This is an important consideration for obstetrical training[49] and has an impact on calf welfare. Appropriate timing is another consideration regarding assisted parturition in cows.[49] On the farm recruited, calving cows are closely monitored and only a small number of experienced staff assist parturition; consequently, early intervention only occurs in cases of malpresentation. It is probable that this approach will have lessened any stress or traumatic injury experienced by cows and calves in this study, and may explain the minimal effect of assistance status.

Volume of colostrum ingested had no effect on calf total plasma protein in this study. Although ingestion of very low volumes of colostrum (less than two litres) has been shown to be associated with failure of passive transfer,[50] a study of nearly 400 farms in America also found that at higher volumes, passive transfer was not affected by the amount of colostrum ingested; timing of colostrum feeding in relation to birth however, was highly significant.[19] It is likely that the lack of effect seen with regard to colostrum volume is due to farm colostrum management protocols. An effect of time and intervention interaction irrespective of assistance status was identified, however, although statistically significant, the clinical significance in this case is questionable as although at 48h postpartum total plasma protein concentration was lower in calves in the ketoprofen treated group, calves in both placebo and the ketoprofen groups had total plasma protein concentration above 5.5g/dL; indicative of adequate passive transfer.[51,52]

Creatine kinase is a muscle specific enzyme[30,53] and plasma concentration would be expected to increase following any muscle damage that might occur at parturition. In contrast to previous studies,[30,31,48] no differences in plasma CK concentration between either cows or calves experiencing assistance and those not experiencing assistance were identified. However, irrespective of assistance status, cows treated with ketoprofen had higher plasma CK concentration at the 24h and 48h sampling points. Intramuscular injections can result in elevated plasma CK concentration due to local muscle trauma at the injection site[53] and a placebo administered by the same route was included in the study design to control for this. A small study of five animals suggested that although intramuscular administration of ketoprofen does not appear to be painful, CK concentration following intramuscular ketoprofen administration is elevated for up to 50h post injection compared to CK concentration in cows administered saline,[54] and this may explain the result seen. Larger injection volumes are associated with increased tissue damage[54] and this may be why a similar effect was not seen in calves in this study. In calves born to primiparous dams, plasma CK concentration was higher than in those born to multiparous dams. Whilst in this study this was not due to an increased likelihood of assistance, it is possible that the smaller pelvic size of primiparous dams[55] results in more tissue trauma to the calf during both assisted and normal parturition.

Intervention alone, or interaction between assistance status and intervention, did not have an effect on any of the biochemical markers measured in cows or calves in this study. Based on these results, the administration of NSAID analgesia to animals following either unassisted or mildly assisted parturition or birth cannot be recommended. However, in all parameters except for cow plasma cortisol concentration there was no effect of assistance status, therefore there is reduced potential for a measurable effect of analgesic intervention. It is possible that if animals experiencing more severe assistance or caesarean section were included in the study, effects of NSAID intervention might have been seen. Additionally, biochemical markers are potentially rather crude indicators of stress and pain in animals[56–58] and subtle welfare impacts may not be detected by these means. Behavioural analysis is widely used for assessing welfare associated with painful events[7,59,60] and can be an indicator of impaired welfare states in situations when biochemical parameters might be expected to be within normal limits. Behavioural analysis of animals reported in this study has been undertaken and will be reported elsewhere. Based on the results reported here, administration of NSAID analgesia to animals experiencing mild assistance or no assistance in the immediate postpartum period does not appear to have beneficial effect on biomarkers of stress and tissue damage.

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Table 1: Scoring system for defining degree of calving assistance. Adapted from Barrier *et al.* (2012, 2013).

Score	Definition	Allocated Group
1	No assistance required or provided	Unassisted
2	Mild farmer assistance – manual pull, no mechanical calving aid used and no repositioning of calf required or performed	Assisted
3	Moderate to severe farmer assistance – Repositioning of calf is performed and/or there is use of mechanical calving aid	Assisted
4	Veterinary assistance – veterinary assisted delivery *per vaginum* or caesarean section required	Excluded from study

Table 2: Exclusion and inclusion of animals for final analysis. Treatment = ketoprofen intervention; placebo = saline intervention. Unassisted = score 1 calving assistance; Assisted = scores 2 and 3 calving assistance grouped together.

Animals excluded from analysis	Reason	*n*	Total
Cows excluded at data collection stage	Illness likely to affect results	2	
	Failure to collect samples	1	
Cows excluded at analysis stage	Mis-recording of intervention or assistance status	2	5
Calves excluded at data collection stage	Congenital umbilical hernia	1	
	Illness necessitating euthanasia for welfare reasons	1	
Calves excluded at analysis stage	Mis-recording of intervention or assistance status	2	4
Animals included in final analysis	**Group**		
Cows included in study	Assisted placebo (AP)	24	
	Assisted treatment (AT)	23	
	Unassisted placebo (UP)	20	
	Unassisted treatment (UT)	22	89
Calves included in study	Assisted placebo (AP)	25	
	Assisted treatment (AT)	22	
	Unassisted placebo (UP)	20	
	Unassisted treatment (UT)	24	91

Table 3: Number of dams recruited from each lactation and proportion of the study population.

Dam lactation	*n*	Proportion of recruited population (%)
1	48	52.7
2	13	14.3
3	15	16.5
4	7	7.7
5	5	5.5
6	2	2.2
7	0	0.0
8	1	1.1

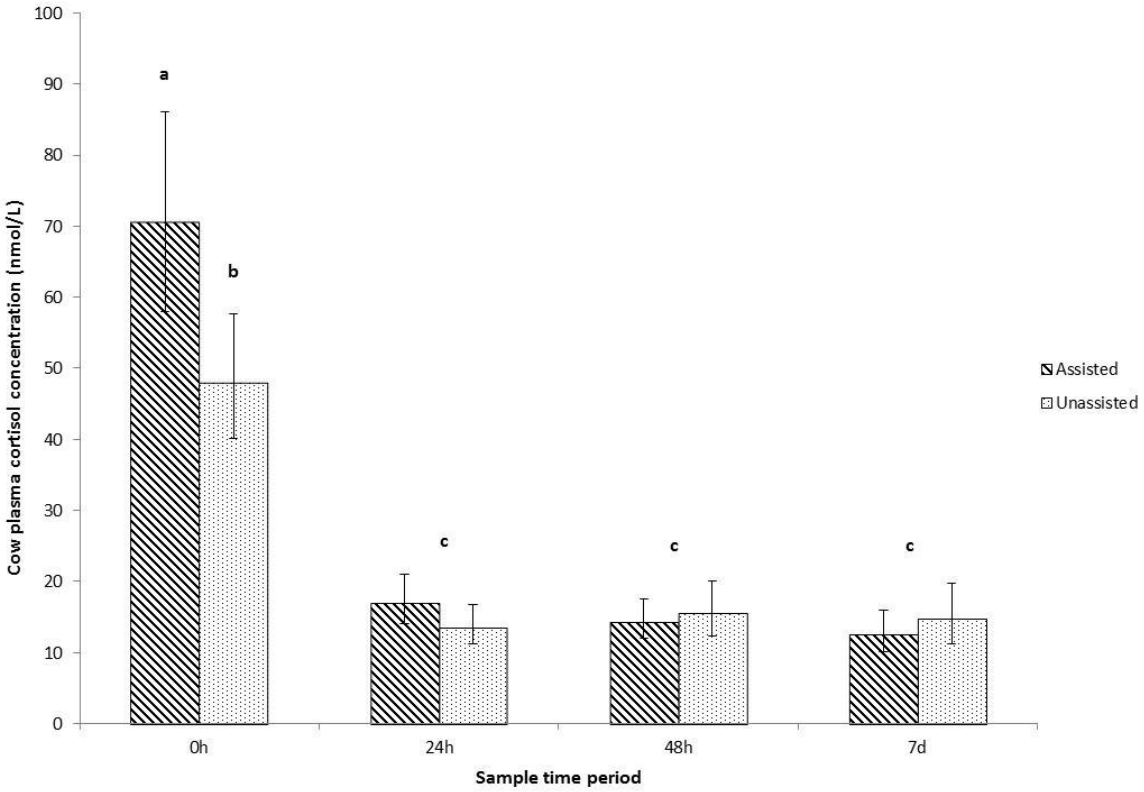

Figure 1: Back-transformed mean and 95% confidence interval (vertical bars) of cow plasma cortisol concentration at each sampling point (time) for cows experiencing assisted parturition (ketoprofen and saline groups combined; *n*=47) and unassisted parturition (ketoprofen and saline groups combined; *n*=42). Different letters indicate significant interaction differences (*p*=0.023).

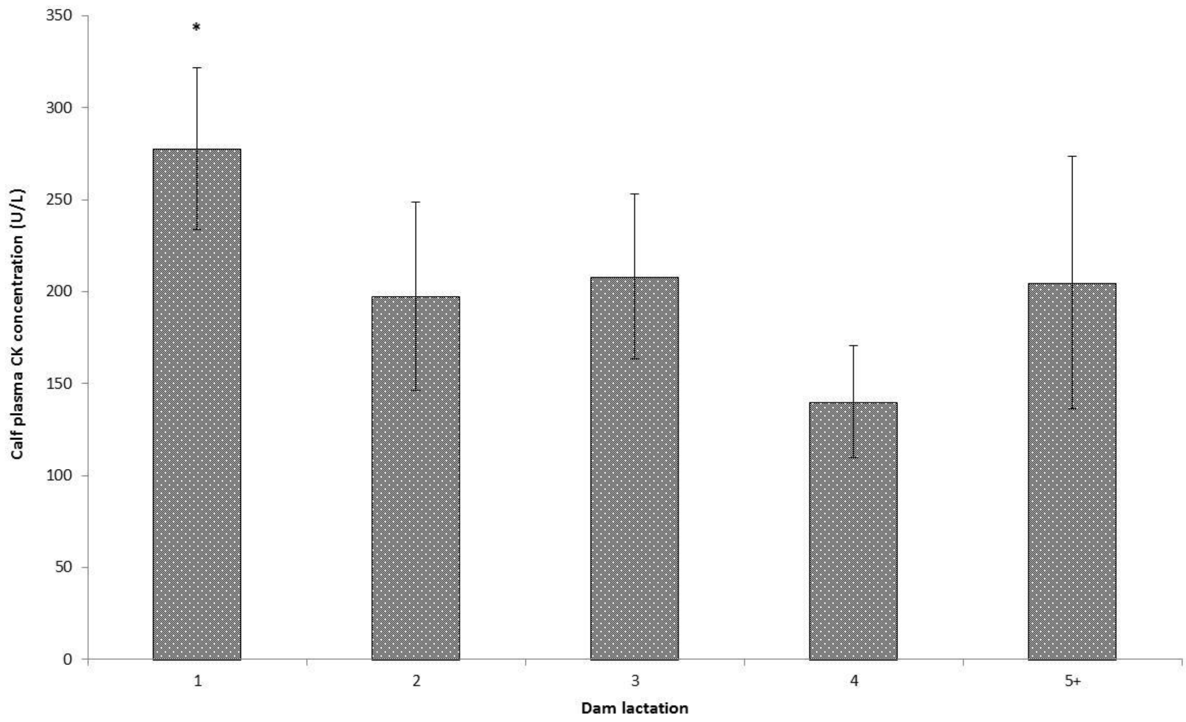

Figure 2: Mean and 95% confidence interval (vertical bars) of calf plasma CK concentration for each dam lactation (all calf groups combined; n=91). *Indicates statistically different compared to other lactations (*p*=0.026). Untransformed data presented to facilitate interpretation.

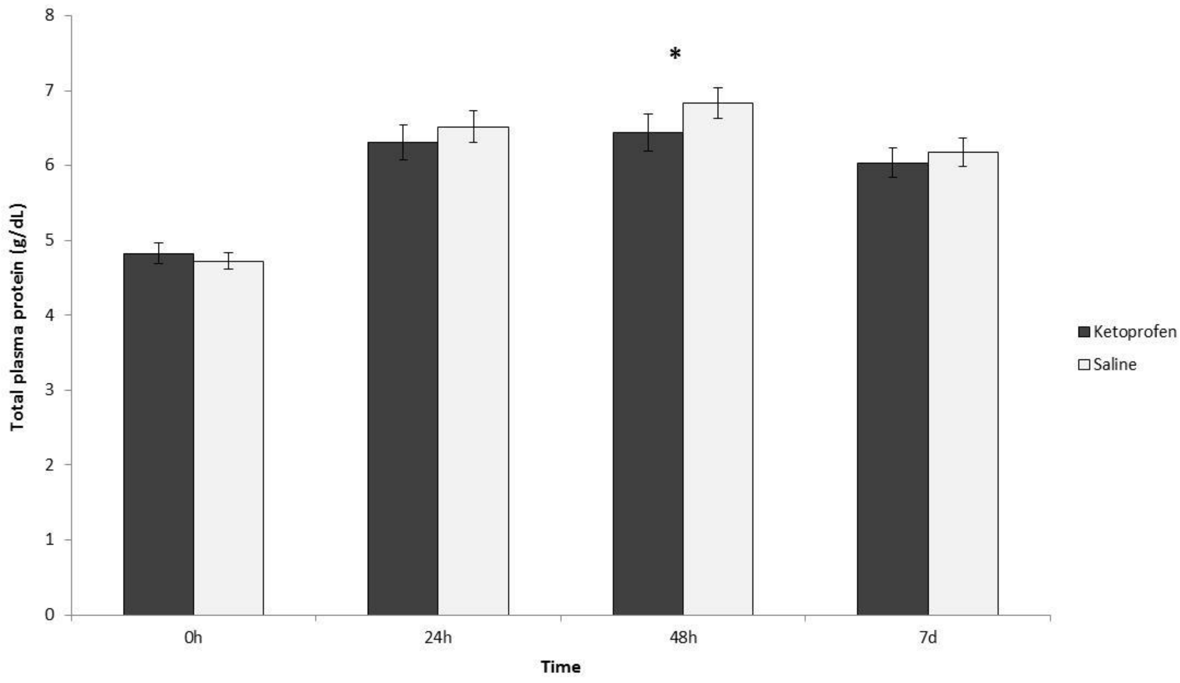

Figure 3: Back-transformed mean and 95% confidence interval (vertical bars) of calf total plasma protein at each sampling point (time) for calves treated with ketoprofen (assisted and unassisted groups combined; *n*=46) or saline (assisted and unassisted groups combined *n*=45). * Indicates significant difference between ketoprofen treated and saline treated calves (*p*= 0.001).