Use of animal behavior and biomarkers for the assessment and monitoring of animal pain during calving

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SUMMARY

Pain is a sensory and emotional experience that reduces the quality of life, causes suffering, and has a negative impact on the economics of farming. Reseant research on animal pain has focused on wildlife, companion, and laboratory animals. In recent years, the welfare of farmed animal species used to produce food has caught the attention of the general public due to the large populations of these animals. Pain in farm animals significantly reduces production and growth rates. However, farm animals do not over-express pain or weakness, therefore recognizing and evaluating pain can be very difficult. Pain is usually associated with routine husbandry practices but normal physiological changes such as calving can also result in pain and stress for both the mother and the newborn. Pain and stress produce similar behavioural responses, these responses are difficult to measure, have marked differences between species and might also differ with similar stimuli.

Calving is an essential feature of the production system that can cause welfare and economic problems. Therefore, it is important to identify pain during calving and appropriately deliver the best physiotherapeutic intervention. Pain assessment tools must be reliable and, allow the assessment and evaluation of pain in a specific manner. In species such as rodents, cows, mares, and sows, current methods to assess pain focus on changes in behavior, physiology, and biological function. These methods include the collection and interpretation of data during different situations such as the administration of analgesics for pain relief. Additionally, specific biomarkers can be used to determine the short and long-term impact of pain on livestock production. Being able to recognize and assess pain will help in the prevention and mitigation of pain in animals and therefore improve animal welfare and production. This review aims to briefly discuss different methods to assess pain during calving and their application to animal production.

KEY WORDS

Pain; behavior; farm animal; calving; biomarker.

INTRODUCTION

In the last decades, there has been a rise in people concerned about animal welfare, as most research on animals is funded by the public, there has been a significant increase on animal pain research. The focus of this research is to guarantee and improve animal welfare. Recent studies have been conducted on companion animals and wildlife. However, livestock animals used to produce food have not received the same attention [1,2,3]. The Farm Animal Welfare Council has developed codes of practice for the care and handling of animals in order to prevent animal suffering [4]. Unfortunately, ensuring that animals are free of pain, injuries or disease is practically impossible. Husbandry practices might result in pain, discomfort, or aversive states that impact psychological functions [5,6]. Additionally, many physiological processes such as calving result in pain [4].

Excessive pain during calving can alter maternal care which significantly reduces the neonate's development and survival rates. Therefore, it is important to identify pain during calving and appropriately deliver the best physiotherapeutic intervention [7,8,9].

The present review focuses on different methods of pain monitoring and assessment during calving. These assessments include animal behavior, biomarkers, description and definition of pain in domestic animals, current research models and evaluation of pain during calving.

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METHODS

Study design

A systematic research of published articles was conducted, to provide an overview of different pain assessment methods. Pain assessment should include the time of evaluation, behavioral observations, and sample collection to measure biomarkers during calving.

Search strategy

This review was carried out according to the Cochrane Collaboration methodology [10]. The development of the protocol was guided by the Preferred Reporting Items for Systematic Review and Meta-Analysis Protocols (PRISMA-P) 2015 statement [11] . Relevant articles were identified by searching on Pubmed and Science Direct using the following keywords: pain and calving. Searches were supplemented by hand searching and retrieval of any additional articles meeting eligibility criteria that were cited in reference lists.

CONCEPT OF PAIN

The International Association for the Study of Pain (IASP) defines pain in humans as: "An unpleasant sensory and emotional experience associated with or resembling that associated with, actual or potential tissue damage" [12]. Unfortunately, the definition of pain in humans cannot be used in animals as it focuses on understanding how animals feel by requiring them to communicate their subjective experiences. Zimmermann (1986) and Sneddon (2009) proposed a new definition of animal pain: "Animal pain is an aversive sensorial experience that results in protective motor actions that modify species-specific behavior traits to avoid pain" [13,14]. Sneddon (2009) added that animals in pain learn rapidly to avoid noxious stimuli and present changes in behavior as a mechanism of defense to reduce future injuries and promote the healing process [14,15]. Molony (1997) defined pain as a sensorial and emotional aversion due to tissue damage. These views of pain have been difficult to integrate but it is important to standardize the concept of animal pain as it is the base to develop an appropiate model for pain assessment.

ANIMAL MODELS FOR PAIN ASSESSMENT

Pain is a complicated process, and as a consequence research on animal pain has been difficult. Studies in animal pain have been affected by different factors such as sex (of the animal and the evaluator), genotype, and social interactions [16]. Although rodent models have been of main relevance for pain research, domestic animals may offer accurate details about human biology and physiology [17]. Humans and pigs share characteristics both anatomically and physiologically, hence the pig could be used as a model for the assessment and treatment of pain in humans[18]. Similarly, the use of other domestic animals might result in a better understanding of pain [17].

Recent studies focus on the following animal reactions to pain:

1. Pseudo-affective reflexes are responses organized by lower motor neurons in the hierarchy of the central nervous system. These reflexes include neurovegetative symptoms (tachycardia, tachypnea, arterial hypertension, etc.), basic motor responses (contractures, withdrawal, etc.), and vocalizations.
2. More complex responses regulated by the upper nervous center, including learned conditioned motor responses such as behavioral modifications (escape, avoidance, aggression, etc.)[19,20].

Both evaluations are unsatisfactory as they might show variations according to the stimuli, plasticity in animals, the methodology used for measurement, and the data analysis [21]. Moreover, we still have difficulties identifying and reducing pain. These include the lack of up-to-date knowledge and poor communication between producers and veterinarians [22].

In summary, it is necessary to create new models and methodology to evaluate pain in different species and production conditions. Additionally, researchers must develop an accurate form of evaluating both physiology and behavior during the pain process to use the information during animal production and experimental procedures. A proper model for the animal pain assessment could improve animal handling during food production and still comply with animal welfare regulations.

BEHAVIOURAL RESPONSES TO PAIN

Pain assessment has two different approaches: the first one evaluates if a procedure results in pain and the efficiency of an analgesic protocol; The second approach determines if an animal is in pain and has received an appropriate analgesic dose. During pain evaluation in animals, both assessments accept a range of variability. However, variability increases between species, especially when random monitoring of pain is required [3].

Pain assessment specific to each species is important to maintain animal production. Previous studies have demonstrated that pain can result in diminished production. In bovine, pain significantly reduces growth rate and milk production [23,24]. Changes in behavior have also been related to pain in domestic animals and they are considered the first indicators of an animal in distress [25].

Using behavior to assess pain is very complex. It requires identifying an abnormal behavior, the circumstances and the possible cause. As a result, changes in behavior are not isolated events, and the context is of major importance [25].

In 2008, Seksel suggested that behavior depends on three main factors:

1. Genetic predisposition.
2. Previous experiences in certain circumstances.
3. The environment where the animal is currently living [25].

Genetic factors may lead to alterations in behavior caused by pain. Predator species present notable variations in behavior when compared to other species. Age is also important, as it might alter the animal's reaction to a certain stimulus or a stressful situation [26,27]. Additionally, the distribution and morphology of opioid receptors might alter the pain signaling cascade and then the pain response [28]. Finally, factors such as familiar or unknown environment, the situation, presence or absence of other animals of the same or different species, the weather, or unknown stimulus may determine the duration and frequency of a certain behavior [29].

HUSBANDRY PRACTICES THAT RESULT IN PAIN

During animal production, different husbandry practices may provoke lesions of healthy tissues like organs, muscles, tendons, bones, nerves, and blood vessels, resulting in chronic or acute pain[30]. Depending on the species, pain is often associated with routine husbandry practices such as castration, tail docking, dehorning, livestock branding, ear tagging or marking, and beak trimming. Additionally, activities such as tattooing, implanting electronic devices, removal of extramammary glands,

orthodontic treatments, nose piercing, crest trimming, phalanx amputation, declawing, and wing clipping, although less common, can result in pain and stress in production animals[30]. An analysis of potential causes for pain in farm animals concluded that diseases, injuries, husbandry practices, and calving are the major causes of pain [5,31,32] .
During calving, factors including the duration of the calving, disproportion in size between the fetus and the pelvis, fetal presentation, vulva stenosis, uterine inertia, uterine torsion, uterine contractions intensity, and cervical stenosis might increase of pain [8,33,34]. Unfortunately, there are no specific protocols for pain assessment during calving which leads to little research on the subject [35].

ASSESSING PAIN DURING CALVING

Gnawing animals

Specific behavior associated with pain has been evaluated in animals that produce multiple offsprings per gestation such as rats and mice[32,36,37]. Pain behavior can include hiding, retraction of pelvic limbs, contraction of the lower abdomen, abdominal distention, and stretching of limbs with stooped posture. Pain behavior might make it difficult for fetuses to pass through the birth canal. However, it has been observed that these behaviors are significantly reduced after morphine epidural injection [37].
Spontaneous behaviors are also reduced in a dose-dependent manner with an infusion of intrathecal morphine if administered one day before delivery[36]. Additionally, behavior associated with pain was reduced in a dose-dependent manner with a systemic infusion of morphine. Morphine sedative effects did not alter normal beaviour such as eating, nesting and caring for the young [32] .
Pain behaviors are also associated with uterine contractions. Uterine contractions start before fetal expulsion, and their frequency and intensity increase with the administration of subcutaneous oxytocin, which increases the signs of pain [37] .

Sows

In sows, there are few studies related to pain during calving. Ison et al. (2016) reported that tremors, a pain-related behavior, significantly increased during the expulsion of the fetus, while no changes were observed in hind limb behavior. Tremors could be the result of accumulative inflammation, pain, or fatigue as labor progresses; consequently, tremors are used as inflammatory pain markers. Additionally, the behavioral changes and their association with the expulsion of piglets might be quantitative indicators of pain[38].
In sows, behavioral scales are used to measure the ease of calving. These scales focus on observations during calvings such as the duration, the position of the sow, and the presence of stillborn piglets and mummified fetuses[39]. These scales demonstrated that primiparous sows present more physical activity when compared to multiparous sows [40].
As for the postpartum pain, Ison et al. (2018) reported that animals present the following behavior: hind limb forward, tremor, and posterior arch, both in primiparous and multiparous sows. However, the ketoprofen administration after calving decreased the arch posture, while forward hind limb and tremors were not significantly affected. Ketoprofen seems to have different effects between primiparous and multiparous sows. Primiparous sows were more stable after the injection of ketoprofen as they presented shorter posterior arch posture and forward behavior of the hind limbs while multiparous sows presented the posterior arch posture after an hour post-injection[39]. Uitdehaag et al. (2008) studied the relation of pain-related behavior during calving in sows with piglet survival at weaning. Sows with high survival at weaning remain seated for longer periods and less time standing when compared to sows with low survival at weaning [41].

Mares

In equines, regardless of its clinical importance, there is limited information about the behavior related to pain, and there is even less information in equine-related species such as donkeys and mules[42]. Aggressive behavior has been related to pain, and this behavior seems to be repetitive under pain conditions[43]. Van Dierendonck et al., (2020) tested and developed two scales for pain: EQUUS-DONKEY-COMPASS y EQUUS-DONKEY-FAP. Both scales include behavior, physiological parameters, response to certain interactions, and facial expressions during painful situations. These scales were test during acute lameness, colic, pain in the head area, and post-surgical pain, but not during calving [44].

Cows

During labor, cows present increased restlessness, tail wagging, and postural changes from rest to standing, such observations are consistent with previous studies [45,46,47].
In cows, dystocia has been directly associated with pain. Studies in Holstein cows demonstrated that pain behavior such as tail lifts are observed more frequently in females with an inadequate calf presentation[48]. Additionally, cows with dystocia show: restlessness at the start of calving, tail lifting, and lying in a lateral position with the head resting longer than cows with eutocic delivering[48] .
These results denote that pain behavior during calving can help identify problems. In addition, laboratory tests can are used to detect the presence of biomarkers that confirm the presence and magnitude of pain during labor and its long-term effects.

PAIN BIOMARKERS

A biomarker is a molecule that can be measurable in an objective, systematic and precise way, in a biological sample. Biomarker concentrations can be established as indicators of normal or pathological processes (Table 1) and be used to monitor the response to treatment[49,50] . Biomarkers can also be used to evaluate the functional or physiological interaction between a biological system and a chemical or physical stimulus [51]. Biomarkers can be measured by molecular biology techniques using DNA, RNA, or protein samples [52].

Sows

In sows, a sudden increase in the cortisol levels during early postpartum has been associated with intrinsic stressful factors associated with pain[53]. In pigs, C-reactive protein and haptoglobin are considered markers of inflammatory lesions[54]. It has been previously reported that high concentrations of haptoglobin and C-reactive protein are present a week after calving indicating an inflammatory process[55]. Additionally, Verheyen et al., (2007) reported that gilt sows have higher levels of haptoglobin when compared to multiparous sows, up to one week after calving [56]. Kostro et al., (2003) demonstrated that C-reac-

Table 1 - Biomarkers around birth in domestic animals.

Biomarker	Species	Evaluation time	Author, year
Cortisol	Cows	Calving	Hydbring et al., 1999 [66].
Progesterone, estrogens	Cows	Days before calving	Zhang et al., 1999 [60].
Cortisol	Cows	Calving day	Patel et al., 1996 [61].
Vasopressin	Cows	Birth	Kendrick et al, 1991[62]; Lawrence et al.,1995[63];
Olsson et al., 1987[65]; Hydbring et al., 1999[66].			
Haptoglobina, suero amiloide A	Cows	Birth	Mainau et al., 2009[67]; Murata et al., 2004[68].
Haptoglobin	Cows	Dystocia	Schönfelder et al., 2005[69].
Cortisol	Sows	Birth	Lawrence et al., 1994[63].
Haptoglobin			
C-reactive protein	Sows	One week postpartum	Kovác et al., 2008[55].
C-reactive protein	Sows	Postpartum	Kostro et al., 2003[57].
Haptoglobin	Sows	One week postpartum	Verheyen et al., 2007[56].
Tumor necrosis factor a			
C-reactive protein	Sows	Six hours postpartum	Ison et al., 2018[39].
IL-6			
IL-1β	Mares	Two hours postpartum	Jaworska & Janowski, 2019[70].

tive protein levels reflect the extent of inflammation of the reproductive tract and mammary glands in sows with mastitis-metritis-agalactia[57]. Stiehler et al., (2016) conducted a study to improve the diagnosis of peripartum hypogalactia syndrome in sows. They evaluated haptoglobin and C-reactive protein levels in blood samples on day seven postpartum and concluded that both proteins are not specific to postpartum disorders. Haptoglobin and C-reactive are related to myometrial activity, posterior train relaxation, and distention of the neck of the uterus [58,56,55]. In primiparous sows, C-reactive protein concentration increased when compared to multiparous sows demonstrating that multiparous sows experience more pain due to uterine activity after parturition, although no evaluation of uterine activity was performed [39]

Additionally, administration of ketoprofen 90 minutes after the expulsion of the last piglet results in a significant increase of the tumor necrosis factor a in multiparous sows when compared to primiparous sows, affecting the levels of protein B, a specific protein of gestation can be used as a marker for fetal well-being in both sows and cows[59].

Cows

In cows, high levels of progesterone and low estrogen concentrations have been associated with dystocia a few days before calving[60]. Cows with two fetuses have a significantly higher concentration of cortisol in plasma on the day of calving when compared with cows with one fetus[61]. This difference in cortisol concentration could be a direct result of the additional stress and trauma experienced by the mother while expelling two fetuses and therefore could be used as a stress marker [48].

Furthermore, heifers that required obstetric maneuvers showed a higher concentration of vasopressin in plasma when compared to animals that did not require obstetric maneuvers. Although the role of vasopressin during labor is still unknown, an increase in concentration at the time of calving has been related to stress[62], intense muscular work[63], an increase in plasma osmolality[64] and/or bleeding[65]. Since vasopressin increases during obstetric maneuvers, it might be associated with muscle work, stress, and pain rather than metabolic effects[66].

Around calving, there are significant changes in the concentration of acute-phase proteins. Haptoglobin and serum amyloid A are markers for pain as they increase around calving due to inflammation or trauma. Additionally, both are significantly higher in heifers when compared to multiparous cows [67,68]. Moreover, Schönfelder et al., (2005) observed higher concentrations of haptoglobin in cows with dystocia after uterine torsion when compared to cows with a eutocic calving [69].

Mares

In mares, biomarkers related to endometritis and retention of the placenta have been evaluated. During placenta retention, there are no significant changes in the expression of pro-inflammatory cytokines and tumor necrosis factor a. On the other hand, the increase of IL-6 expression in the allantochorion and IL-1b in the endometrium could reflect a local immune response that leads to detachment of the fetal membranes demonstrating an inflammatory response linked to parturition in mares [70].

IMPLICATIONS

In summary, pain research around calving in domestic animals requires the integration of new technologies to establish biomarkers and pain-related behavior by species. These methods for the assessment of pain could be applied to livestock productions by veterinary doctors and workers to improve the reproductive care of females. On that account, it is necessary to develop specific protocols to improve pain estimates in animal models[71].

Behavioral evaluations to identify and alleviate pain in females during parturition must be carried out in farm conditions. In the case of childbirth, observations and sampling must be done during the expulsion phase of the fetuses and, if necessary, follow-up during the postpartum to establish treatments and therapeutics suitable for each species to reduce pain.

CONCLUSIONS

Studies regarding pain around calving in domestic animals have yielded results that have created the basis of pain evaluation during animal production. However, to obtain behavioral patterns and determine specific biomarkers related to pain in domestic species, it is necessary to identify the needs and characteristics of each species and use technology aimed at this purpose. Additionally, calving observation should take place in real situations and with the proper tools that allow researchers to obtain results objectively and effectively. These should also include different animal management during calving so that the results can be used to improve animal welfare.

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