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CLINIC AND SURGERY

The analgesic effect of preventive administration of meloxicam in calves submitted to hot-iron dehorning

Guilherme Trevisan[1] Renata Haddad Pinho[2] Jackson Barros do Amaral[3] Stelio Pacca Loureiro Luna[1*]

[1]Departamento de Cirurgia Veterinária e Reprodução Animal, Faculdade de Medicina Veterinária e Zootecnia (FMVZ), Universidade Estadual Paulista (Unesp), 18618-681, Botucatu, SP, Brasil. E-mail: stelio.pacca@unesp.br. *Corresponding author.
[2]Departamento de Especialidades Cirúrgicas e Anestesiologia, Faculdade de Medicina de Botucatu (FMB), Universidade Estadual Paulista (Unesp), Botucatu, SP, Brasil.
[3]Centro de Análises e Pesquisas Tecnológicas dos Agronegócios de Bovinos de Leite (CAPTA) Instituto de Zootecnia de Nova Odessa, Nova Odessa, SP, Brasil.

ABSTRACT: *Dehorning is a zootechnical practice that causes severe pain in cattle. Although there are several studies evaluating the effects of analgesics in calf dehorning, none of them used validated pain assessment instruments. We evaluated the analgesic effectiveness of meloxicam administered before dehorning, compared to a control group, using the Unesp-Botucatu, numerical, simple descriptive, and visual analogue scales for pain assessment before and 4, 8, and 24 hours after the dehorning in 44 female calves. All calves received 0.04 mg/kg of xylazine IM 20 minutes before dehorning and local anesthetic block with 2% lidocaine with a vasoconstrictor. Calves were divided into two groups: without (GX; n = 22) or with 0.5 mg/kg of meloxicam (GXM; n = 22) administered intravenously before the procedure. Dehorning was performed through the section of the base of the horn bud, followed by thermocautery disbudding. For comparisons over time, mixed linear or generalized mixed linear model were used. The interaction between groups and study phases was used as fixed effects and each calf as a random effect. Bonferroni post hoc test was used. There was an increase in the pain scores at 4h compared to baseline in both groups (GX and GXM) for the four scales. The scores at 4h were higher in GX compared to GXM for all scales. Meloxicam reduced, but did not eliminate, behavioral expressions of pain in calves submitted to hot-iron dehorning. Therefore, it should be included in the analgesic protocol to improve welfare in calves undergoing dehorning.*
Key words: *animal welfare, cattle, meloxicam, pain.*

Efeito analgésico da administração preventiva de meloxicam em bezerros submetidos à descorna com ferro quente

RESUMO: *A descorna é uma prática zootécnica que causa dor intensa em bovinos. Há na literatura diversos estudos sobre os efeitos de analgésicos para mitigar a dor frente a descorna, mas nenhum usando escalas validadas. Avaliamos a eficácia do meloxicam administrado previamente à descorna, comparado a um grupo controle, utilizando-se as escalas Unesp-Botucatu, numérica, simples descritiva e analógica visual para avaliação da dor antes e 4, 8 e 24 horas após a descorna em 44 bezerros fêmeas tratadas com 0,04 mg/kg de xilazina IM 20 minutos antes da descorna e bloqueio anestésico local com lidocaína a 2% com vasoconstritor. Os bezerros foram alocados em dois grupos: sem (GX; n=22) ou com 0.5 mg/kg de meloxicam (GXM; n=22) administrado por via intravenosa antes do procedimento. Realizou-se a descorna por secção da base do botão cornual seguido de termocauterização. Para as comparações ao longo do tempo, empregou-se o modelo linear ou linear misto. Considerou-se a interação entre grupos e momentos como efeito fixo e cada bezerro como efeito aleatório. As alterações foram inferidas de acordo com o pós-teste de Bonferroni. Para as quatro escalas houve aumento dos escores às 4h comparado ao basal em ambos os grupos (GX e GXM). Os escores de todas as escalas às 4h foram maiores em GX que em GXM. O meloxicam reduziu, mas não aboliu, a expressão comportamental da dor em bezerros submetidos à descorna com ferro quente, o que sugere o uso de terapia antálgica multimodal para realizar tal procedimento e garantir o bem-estar animal.*
Palavras-chave: *bem-estar do animal, bovinos, dor, meloxicam.*

INTRODUCTION

Despite the growing concern for animal welfare, pain control in farm animals is still neglected (CANOZZI et al., 2020; HEWSON et al., 2007; HUXLEY & WHAY, 2006; LORENA et al., 2013). Until recently, this was partly due to the lack of precise instruments to assess pain (DOCKWEILER et al., 2013). In cattle, several zootechnical practices culminate in acute perioperative pain that can become

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chronic or neuropathic if preventive measures are not taken. The most common procedures that cause pain in this species are branding, castration, and dehorning (CANOZZI et al., 2020). The use of a hot iron when dehorning calves is still a widespread practice among breeders and is frequently used in the field. This procedure is one of the leading causes of suffering in dairy calves (GOTTARDO et al., 2011).

Several techniques can be used to mitigate pain during dehorning (STEWART et al., 2009). The isolated application of local anesthesia is apparently insufficient, given its temporary effect and the complexity of innervation, due to the intimate contact of the horn with the bone structure. The simultaneous use of non-steroidal anti-inflammatory drugs (NSAID) is demanded to minimize a delayed cortisol increase and nociceptive and behavioral pain signals (MILLIGAN; DUFFIELD; LISSEMORE, 2004; PETRIE et al., 1996). Hence, the association of local anesthesia and non-steroidal anti-inflammatory drugs is recommended for pain relief when dehorning calves to inhibit nociception and inflammation, respectively (STOCK et al., 2013). Another important drug used during dehorning is xylazine. Its myorelaxant effect produces decubitus and chemical restraint to facilitate positioning during the procedure (CARAY et al., 2015) and reduces behavioral and physiological indicators of pain in calves subjected to dehorn (REEDMAN et al., 2021). In this context, the association of meloxicam with xylazine appears to be an effective analgesic strategy (HEINRICH et al., 2010).

The act of assessing pain requires instruments that guarantee the reliability and validity of the results to assist in decision-making regarding the need for analgesics (BRONDANI et al., 2013). Although the analgesia provided by meloxicam has been studied in calves submitted to dehorning, pain scales were not used to perform the assessment (HEINRICH et al., 2010). For the bovine species, composite pain scales based on behavior (OLIVEIRA et al., 2014) and facial expressions are available for adults (GLEERUP et al., 2015). However, none of these instruments has been used to assess pain in calves after dehorning. In addition to intensity, composite scales measure the sensory and emotional sensations (MURRELL et al., 2008). Unidimensional scales such as the Simple Descriptive, Numerical, and Visual Analogue scales are also used to assess pain in this species (OLIVEIRA et al., 2014); however, they represent only the intensity of pain and have less repeatability and reproducibility compared to composite scales.

The current study evaluated the effectiveness of the preventive use of meloxicam as an analgesic in calves subjected to hot-iron dehorning by measuring the pain scores of the Unesp-Botucatu cattle pain scale (UCAPS), numerical (NS), simple descriptive (SDS), and visual analogue (VAS) scales.

MATERIALS AND METHODS

Facilities and animals

The study was carried out at the Dairy Cattle Research Center of the Zootechnics Institute of Nova Odessa, of the Secretariat of Agriculture and Supply SAA of São Paulo. For this, 44 female dairy calves were used, with ages of approximately 60 ± 15 days and weights of 70 ± 19 kg.

As an inclusion criterion for the experiment, the animals were considered healthy when they did not present clinical alterations in the inspection of the mucosa and episcleral vessels, skin turgor, heart and respiratory rates, and rectal temperature.

The weaned calves were allocated to two groups of 22 animals denominated GX (xylazine) and GXM (xylazine and meloxicam). They were managed in collective hutches, in shelters with feed of chopped hay and concentrated feed, mineral supplementation, and water *ad libitum*, in addition to artificial feeding provided in the morning and afternoon. During the experiment, the humane management of animals was considered.

Anesthetic protocol and groups

The animals of both groups received 0.04 mg/kg of xylazine administered to the semitendinosus or semimembranosus muscle (IM) 20 minutes before dehorning. Five minutes before the beginning of the dehorning local anesthetic block (7 mg/kg of 2% lidocaine hydrochloride with a vasoconstrictor) was performed. Half of the dose was injected by perineural infiltration in the cornual nerve and the other half was injected in separate points surrounding each horn bud.

For the GXM group (n=22), in addition to the previous protocol, 0.5 mg/kg of meloxicam was administered intravenously through the marginal ear vein with a butterfly needle (19G), concomitantly with the administration of xylazine IM.

For the GX group (n=22), there was no administration of meloxicam prior to the procedure. Only animals of the GX group received ketoprofen (3 mg/kg) and morphine (0.2 mg/kg) intravenously through the marginal ear vein with a butterfly needle (19G) to guarantee adequate postoperative analgesia after the first postsurgical pain assessment.

Dehorning

In the afternoon of the day before the dehorning, the animals were housed in collective pens. Shaving was performed using a shearing machine and food and water fasting were instituted for twelve hours because the calves were already ruminating.

Between 8 and 9 AM the following morning, each animal was taken to the restraint stock, where it was maintained in a quadrupedal position, with the cervical region restrained. Antisepsis was performed with iodized alcohol around the horn region. During the procedure, the animal's head was held in manual restraint by an assistant to avoid injuries and burns during the section of the horn bud. Dehorning was performed five minutes after the anesthetic block, through the section at the base of the horn bud, followed by thermocautery disbudding with a burning iron for 30 seconds continuously.

Pain assessment

An evaluator blinded to the treatments was responsible for all in-person assessments outside the observation paddock. He had two years experience with cattle management. The scales were evaluated in the following order: the unidimensional numerical scale (NS) (ordinal numbers from 1 - no pain to 10 - worst possible pain), simple descriptive scale (SDS) (1 - no pain, 2 - mild pain, 3 - moderate pain and 4 - severe pain), visual analogue scale (VAS) (a line of 100 mm, where 0 mm represents no pain and 100 mm the worst possible pain) and the modified UCAPS (0-15) (Table 1). Pain assessment was performed for 5 minutes. The animals were evaluated at the following moments: before the dehorning (baseline) and 4, 8, and 24 hours after the dehorning.

Unesp-Botucatu cattle pain scale (UCAPS)

For the behavioral assessment of pain, the behaviors described in the Unesp-Botucatu scale to assess pain in cattle were used (OLIVEIRA et al., 2014), but modified by including behaviors described in the literature as relevant to assess pain after dehorning (CURRAH; HENDRICK; STOOKEY, 2009; MILLMAN, 2013; WATTS & STOOKEY, 2000). These behaviors were: shake the head and flick the ears (FAULKNER & WEARY, 2000; HEINRICH et al., 2010; STOCK et al., 2013) and scratch the wound (ADCOCK et al., 2020; CUTTANCE et al., 2019).

Statistical analysis

Statistical analysis was performed in R software with the RStudio integrated development environment (Version 4.1.0 [2020-06-22], RStudio, Inc.). The functions and packages were presented in the format 'function{package}' and the significant level of 5% was considered in all tests.

Intragroup changes overtime (baseline vs 4 h vs 8 h vs 24 h) and differences between groups (GX vs GXM) at each moment were analyzed by mixed linear model ('lmer{lme4}') for data showing Gaussian model residual ('resid{stats}') distribution (VAS) according to quantile-quantile plot ('qqnorm{stats}') and Shapiro-Wilk test ('shapiro.test{stats}'). For data with non-normal distribution (NS, SDS, and UCAPS) the generalized mixed linear model ('glmer{lme4}') was applied. The interaction between groups and time-points was used as a fixed effect and each calf as a random effect for all models. Multiple comparisons were assessed by the Bonferroni post hoc test ('emmeans{emmeans}' and 'cld{multcomp}').

RESULTS

There were no complications and/or exclusions of animals during the study. The numerical results are shown in table 2 and figure 1. For the four scales, there was an increase in scores at 4 h compared to baseline in both groups (GX and GXM). Only GX animals presented a reduction in pain scores at 8h compared to 4h. At 24h, the scores did not differ from baseline on any scale in any group. The scores at 4h were higher in GX compared to GXM for all scales.

DISCUSSION

All pain assessment scales were responsive to the painful procedure, culminating in increased scores after dehorning for both groups. Preventive analgesia with meloxicam minimized postoperative pain.

The effectiveness of anti-inflammatory drugs to prevent pain after dehorning is controversial. Some studies have shown pain mitigation when administering meloxicam (ALLEN et al., 2013; HEINRICH et al., 2009) or ketoprofen (MILLIGAN; DUFFIELD; LISSEMORE, 2004; MILLS et al., 2020). Conversely, other studies have reported no differences between groups that received or did not receive NSAID (COETZEE, et al., 2012; HUBER et al., 2013; KLEINHENZ et al., 2017). Although differences in pain scores were observed in the present study between animals submitted to dehorning and treated with NSAID compared to untreated animals, the anti-inflammatory did not completely abate the pain.

Table 1 - Unesp-Botucatu Modified Pain Scale (UCAPS).

Behavior	Quantification	
Vocalization	(0) Absent (1) Present	
Shake the head	(0) Absent (1) Present	
Flick the ears	(0) Absent (1) Present	
Scratch the surgical wound	(0) Absent (1) Present	
Head position	(0) Head above the line of spinal column (1) Head below the line of spinal column	
Interactive behavior	(0) Active; attention to tactile and/or visual and/or audible environmental stimuli; when near other animals, can interact with and/or accompany the group. (1) Apathetic: may remain close to other animals, but interacts little when stimulated	
Activity	(0) Moves normally (1) Restless, moves more than normal, lies down and stands up with frequency or moves less frequently in the pasture or only when stimulated	
Appetite	(0) Normorexia (1) Anorexia	
Rumination	(0) Absent (1) Present	
Locomotion	(0) Walking with no obviously abnormal gait (1) Walking with restriction, may be with hunched back and/or short steps or is reluctant to stand up, standing up with difficulty or not walking.	
Miscellaneous behaviors	Wagging the tail abruptly and repeatedly.	(0) Absent (1) Present
	Extends the neck and body forward when lying in ventral recumbency.	(0) Absent (1) Present
	Moves and arches the back when in standing posture.	(0) Absent (1) Present
	Kicking/foot stamping	(0) Absent (1) Present
	Lying down in ventral recumbency with full or partial extension of one or both hind limbs.	(0) Absent (1) Present

The previously cited studies did not use validated tools to assess pain, making comparisons of reproducibility difficult. The UCAPS was modified and complemented by specific behavioral indicators of pain related to dehorning, so this tool has not yet been validated in its modified version. Validated scales are ideal to accurately assess pain in animals (COETZEE, 2013). Behavioral analysis (FORDYCE; MCMILLAN; MCGRATH, 2018; LAUDER et al., 2020) and validated pain scales based exclusively on pain behaviors (OLIVEIRA et al., 2014) or even associated with facial expressions (GLEERUP et al., 2015; MÜLLER et al., 2019) have already been developed to assess pain in bovine species. However, there are no validated tools for pain assessment in young calves like the animals used in the current study or for the dehorning procedure.

To our knowledge, for ethical reasons, although dehorning should be performed in calves as young as possible to minimize the pain impact, there is no consensus about the best age for this procedure. Hyperalgesia and allodynia were not minimized when dehorning was performed in three-day-old calves compared to 35-day old ones. Actually, allodynia is more evident in younger than older calves for up to 9 weeks after dehorning even with the administration

Table 2 - Median (minimum-maximum) of the UCAPS, NS, and SDS and mean VAS (standard deviation) before and after dehorning of calves subjected to sedation with xylazine and local block and treated with meloxicam before dehorning IV (GXM; n = 22) or with ketoprofen and IV morphine 5 h after dehorning (GX; n=22).

		----------Moments----------			
Scales	Groups	Baseline	4h	8h	24h
UCAPS	GX	0 (0-4)^{c}	10 (0-14)^{aA}	0 (0-13)^{b}	0 (0-7)^{bc}
	GXM	0 (0-4)^{c}	9 (0-13)^{aB}	2 (0-12)^{b}	0 (0-13)^{c}
NS	GX	2.5 (1-5)^{bc}	8.5 (1-10)^{aA}	3.5 (1-10)^{b}	2.5 (1-4)^{c}
	GXM	2 (1-5)^{c}	5.5 (1-10)^{aB}	3.5 (1-9)^{ab}	2.5 (1-9)^{bc}
SDS	GX	1 (1-2)^{b}	3.5 (1-4)^{aA}	1 (1-3)^{b}	1 (1-2)^{b}
	GXM	1 (1-3)^{c}	2 (1-4)^{aB}	2 (1-4)^{ab}	1 (1-3)^{bc}
VAS	GX	3.4±1.6^{c}	7.9±2.3^{aA}	5.4±2.5^{b}	4.4±1.8^{bc}
	GXM	3.1±1.8^{b}	6.6±2.8^{aB}	5.6±2.5^{a}	3.9±1.6^{b}

UCAPS - Unesp-Botucatu Cattle Pain Scale, NS - Numerical Scale, SDS - Simple Descriptive Scale, VAS - Visual Analogue Scale. Intragroup differences between time-points are expressed in lower letters ($^{a}>^{b}>^{c}$) and intergroup differences for each time-point are indicated by upper letters ($^{A}>^{B}$) according to the multiple comparisons in the post hoc test with Bonferroni correction based on mixed linear model (VAS) and generalized mixed linear model (UCAPS, NS, and SDS).

of meloxicam and local blockade (ADCOCK & TUCKER, 2018). The UCAPS, originally developed to assess pain after orchiectomy, was adapted for dehorning and for younger animals, since pain behaviors vary according to age (PICKERING et al., 2006). The evaluated behaviors included those described in the Unesp-Botucatu Scale (OLIVEIRA et al., 2014) and other studies (ADCOCK et al., 2020; CURRAH et al., 2009; CUTTANCE et al., 2019; FAULKNER & WEARY, 2000; HEINRICH et al., 2010; MILLMAN, 2013; STOCK et al., 2013; WATTS & STOOKEY, 2000) and were apparently important for assessing pain in calves, given the responsiveness of the instrument (increase in scores after the painful stimulus, followed by a reduction at 24 hours).

In the present study, the first postoperative pain assessment was 4h after the procedure based on the previous study that validated the UCAPS. According to a recent study, maximal pain intensity after dehorning occurred within 3h after the procedure, followed by reduction of cortisol and pain behaviors, especially in calves treated with local blockade and NSAID compared to calves not receiving analgesia (REEDMAN et al., 2020). However, in our study, differences in pain scores were still observed even at 4h after dehorning. Despite being a common practice in cattle breeding, there is evidence that dehorning causes a level of pain that does not corroborate with animal welfare practices. With regard to animal production, the non-use of analgesics to carry out such zootechnical practices can culminate in less weight gain and financial loss (SAAG et al., 2018; TELLES et al., 2016).

The administration of NSAIDs (ketoprofen) and opioids (morphine) 5 hours after dehorning reduced GX pain scores at 8 hours after surgery. The rationale for providing this rescue analgesic protocol was to minimize suffering and improve postoperative welfare. However, this analgesic intervention made it difficult to compare pain scores between groups at 8h and 24h after dehorning. Otherwise, the positive contribution of this result was that the postoperative pain scores after ketoprofen and morphine were similar to the preventive administration of meloxicam, suggesting that the inclusion of opioids in conjunction with NSAIDs mitigate pain after dehorning in calves.

Some limitations can be attributed to this study. Although the observer was blinded to the experimental groups, because the evaluations were performed face-to-face and not remotely by video analysis, it was not possible to blind the evaluator to the time-points, which may have generated a tendency for the evaluator to overestimate the scores in the postoperative period, compared to baseline. In the same sense, the evaluator's presence may have altered or inhibited the pain expression behavior of animals, as observed in laboratory species (PINHO et al., 2020; SORGE et al., 2014). However, both groups were evaluated in equal conditions and these circumstances are inherent of a field study and real-world situation. Another limitation was the possible residual sedative effect of xylazine falsely increasing

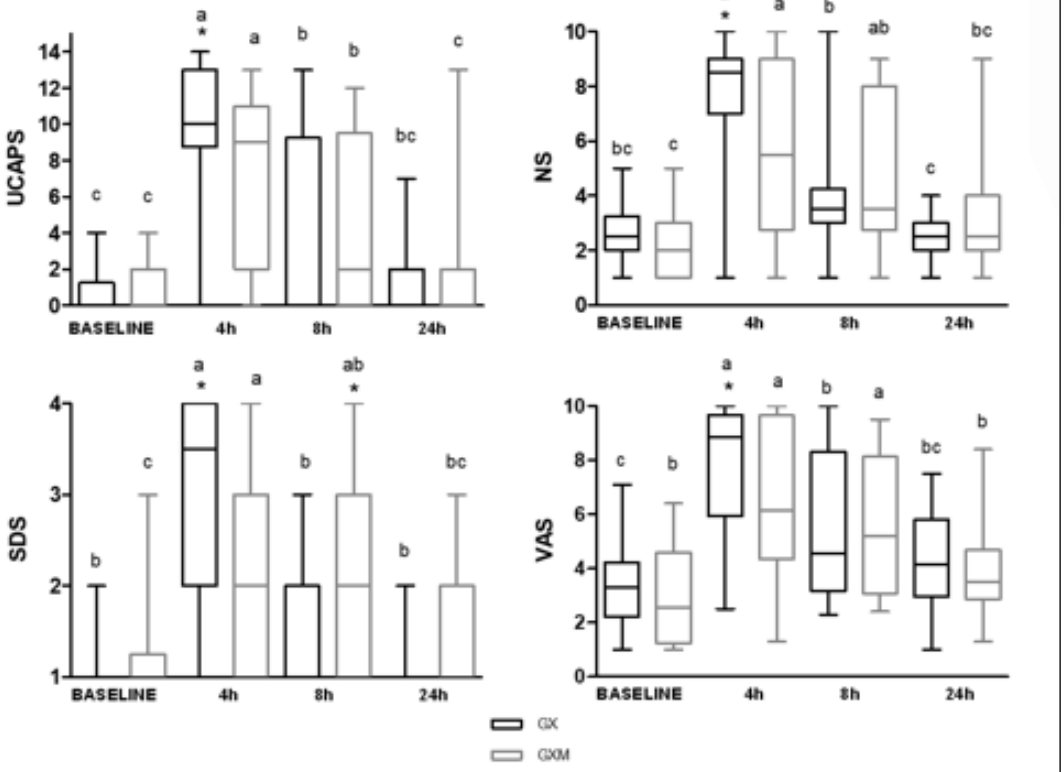

Figure 1 - Boxplots of the scores of the UCAPS, NS, SDS, and VAS scales before and after dehorning of calves subjected to sedation with xylazine and local block and treated with meloxicam before dehorning IV (GXM; n = 22) or with ketoprofen and IV morphine 5 h after dehorning (GX; n=22).

The top and bottom box lines represent the interquartile range (25 to 75%), the line within the box represents the median, and the extremes of the whiskers represent the minimum and maximum values. UCAPS - Unesp-Botucatu Cattle Pain Scale, NS - Numerical Scale, SDS - Simple Descriptive Scale, VAS - Visual Analogue Scale. Intragroup differences between time-points are expressed in lower letters ($^{a}>^{b}>^{c}$), and * indicates intergroup differences at each time-point according to the multiple comparisons in the post hoc test with Bonferroni correction based on mixed linear model (VAS) and generalized mixed linear model (UCAPS, NS, and SDS).

pain scores (REEDMAN et al., 2020); however, we believe that by 4h postoperatively, the effect of xylazine had been abated, as reported in the study that validated UCAPS (OLIVEIRA et al., 2014).

CONCLUSION

Preventive administration of meloxicam and postoperative ketoprofen combined with morphine reduced, but not eliminated, behavioral expression of pain in young calves subjected to hot-iron dehorning. Non-steroidal anti-inflammatory drugs should be included in the analgesic protocol to improve welfare in calves undergoing dehorning.

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BIOETHICS AND BIOSECURITY COMMITTEE APPROVAL

The current study was approved and registered in protocol 246-17 by the Ethics Committee on the Use of Animals (CEUA) of the Animal Science Institute (IZ), of the Secretariat of Agriculture and Food Supply of the State of São Paulo-SAA/SP. The experiment was conducted at the headquarters of this Institute, in Nova Odessa - SP, next to the Research Center for Dairy Cattle.

DECLARATION OF CONFLICT OF INTEREST

The authors declare no conflict of interest. The funding sponsors had no role in the study's design, data collection, analysis, or interpretation, manuscript writing, and the decision to publish the results.

AUTHORS' CONTRIBUTIONS

All authors contributed equally to the conception and writing of the manuscript. All authors critically revised the manuscript and approved the final version.

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