Assessing the Effect of Pain Relieving Methods During Caustic Disbudding Procedure on Dairy Calf Welfare

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ABSTRACT

Dehorning and disbudding are considered painful yet important routine husbandry procedures in the global dairy industry. There are several methods of dehorning and disbudding. In Israel, it is common to disbud calves at a few days to one week of age, using a mainly caustic paste, which produces a chemical burn of the horn buds. The use of local analgesics and systemic analgesics or a combination of the two could assist calves in handling the painful procedure for the hours following. In the study, we examined the influence of local anesthesia in two different age groups, on calf welfare. In addition, the influence of systemic analgesics was examined in the group of older calves, since there is no medical indication for the usage of non-steroidal anti-inflammatory drugs (NSAIDs) in calves below the age of one week. The behavior of the calves was monitored before and during the hours following disbudding, along with blood cortisol levels. Results of this study showed that the age of the calf when undergoing the disbudding procedure influenced welfare. It is not entirely clear whether older calves exhibit signs of pain more clearly, since in the behavioral criteria for calf welfare that was examined in this research, the older group exhibited greater signs of pain, whilst there was no significant difference in blood cortisol levels between the two age groups. Yet, results also showed that when treated with local anesthesia along with systemic analgesia, blood cortisol was lowered more efficiently than shown in all treatment groups. In addition, head-shaking behavior was significantly less frequent compared to all other treatment groups. Thus, the results of this study indicate that there is justification for use of analgesia during the disbudding procedure in common dairy farm husbandry in Israel. In light of this justification, the disbudding procedure should preferably be performed at the age of 8-14 days, since below the age of 7 days there is no medical indication for use of NSAID's anti-inflammatory drugs.

Keywords: Welfare; Disbudding; Caustic Paste; Pain; Activity; Stress; Cortisol; Calves.

INTRODUCTION

While most animal mutilation is gradually seceding (1), disbudding, a painful yet essential routine husbandry procedure, is still practiced in the global dairy and beef industries (2). There are various methods to prevent horn growth (3-4). Disbudding, the disruption of the corium, and the surrounding tissue is carried out using three main methods, which include heat cauterization, chemical cauterization, and scooping (5). Comparing caustic paste disbudding with heat cauterization, reveal longer-lasting signs of pain (6). The extent of pain and stress-induced at the time of disbudding is usually measured by physiological and behavioral means (7, 8).

Behavioral changes following disbudding are one of the

main observed indicators of stress and pain (4, 9). Their typical movements such as head shaking and jerking, ear flicking, leg stamping and kicking are indicative of severe stress and pain in calves (10). Additional behavioral patterns are frequent walking and restlessness, shorter duration of rest and decreased rumination (11). All of the above mentioned behavioral changes can indicate negative arousal, reflecting the mental state of the calf (12).

One of the main physiological indicators for stress response is blood cortisol (13), a glucocorticoid hormone excreted from the adrenal gland in a cyclic pattern as well as in response to stress or pain (14). At an advanced age, disbudding using caustic paste or cauterization and dehorning by horn amputation have an increased effect on the cortisol plasma concentration (15). A cortisol secretion pattern has previously been shown following dehorning or disbudding (16). Cortisol concentration begins to rise immediately after the procedure, reaching maximum levels approximately 30 minutes later and decreasing gradually until reaching normal levels 6-8 hours following the procedure (17, 18).

The use of local anesthesia for caustic paste disbudding has been disputed over the years for benefit while investigating the accurate pain relief for this procedure (19, 20). Three hours after application caustic paste disbudding, still appears acutely painful. The use of a cornual nerve block was effective while local anesthetics were not (21). In this research, we evaluated the different pain relief methods on the calves' behavior following 8 hours post-caustic paste disbudding.

MATERIALS AND METHODS

Animals and treatment groups

The study was conducted in a dairy farm of "Gvulot" located in the southern desert area of Israel. The research project received the approval of the Agricultural Research Organization Ethics Committee.

The research population was composed of 112 nursing dairy calves and the study took place over eight weeks. Once a week, a group of 16 calves was randomly chosen, all calved normally without human intervention and without twin births. All were declared healthy by a certified veterinarian, with no clinical illness before entering the study. The calves were housed individually in pens 2.5 X 1 meters on dry sand bedding.

The calves in the treatment groups underwent a disbudding procedure and were randomly assigned to the different treatment groups. The treatment groups were composed of two age groups with different treatments:

Young group – composed of 40 calves of 1-7 days of age. In this age group, the calves were randomly assigned to three treatment groups. The first treated with a local anesthetic before disbudding (y-lid), the second a control group which did not receive any local anesthetic (y-cont), and was disbudded without any means of pain relief. A third group which was the control and did not undergo any disbudding procedure (Naïve).

Older group – composed of 72 calves 8-14 days old. In this age group, the calves were randomly assigned to five treatment groups. The first was treated with local anesthetic (o-lid), the second received systemic analgesia (o-NSAIDs), the third received a combination of the two (o-lid+NSAIDs). The fourth was disbudded with no medical treatment (o-cont) and the fifth was the control and did not undergo any disbudding procedure (o-naïve).

Local anesthesia 4 ml of lidocaine (Lidocaine HCL 2%®; containing lidocaine hydrochlorine 20 mg/mL and epinephrine 0.01 mg/mL, Bimeda Canada, Ontario, Canada) was injected subcutaneously to the cornual nerve halfway between the eye and the horn bud, perpendicular to the head. The systemic analgesia administered was 2 ml meloxicam (®Loxicom 20 mg/ml, Norbrook Laboratories Ltd., Monaghan, Ireland), a non-steroidal anti-inflammatory drug injected subcutaneously into the lateral area of the neck.

For disbudding, a thin layer of caustic paste containing 24.9% sodium hydroxide and 37.8% calcium hydroxide (Dehorning paste, Dr. Naylor, H.W. Naylor company, Inc., Morris, N.Y., U.S.A.) was administered bilaterally locally on the horn bud.

Sampling and Analysis

To assess the pain and stress occurring during disbudding and during the following hours, behavioral and physiological parameters were evaluated.

Twenty-minute video recordings were taken at set times to assess calf behavior. They were taken before disbudding and then at one, two, three, four, six, and nine hours post disbudding. Calf behavior was analyzed by the same person to avoid subjective differences in the behavior analysis, and

the analysis was performed blindly without disclosure of the treatment administered in each video. The behaviors analyzed in the video recordings were head shaking and head rubbing (11). The intensity of head-shaking was tested by counting the numbers of head shakings recorded by video, every hour following disbudding (22).

Padometry sensors – additional behavioral parameters were measured by leg sensor tags (Afiact®, Afimilk, Kibbutz Afikim, 1514800, Israel) placed on the calves' metatarsus at the beginning of each day of the study. The parameters measured with the sensor tags were the number of steps taken, number of recumbency changes (from lying to a standing position and vice versa), and time spent lying down. Data from the sensor tags was taken at the beginning of each video session.

Physiological response to pain was measured by measuring plasma cortisol levels (13). Blood was sampled from the calves at set times: before disbudding and then at one, four, and eight hours post disbudding. Blood was centrifuged at 2000 RPM for 10 minutes. The serum was separated and frozen at -20°C. The samples were analyzed for plasma cortisol concentration using an ELISA kit (DRG Instruments GmbH, Marburg, Germany). The change in blood cortisol levels is presented as the ratio between the cortisol concentrations measured 1, 4, and 8 hours post-disbudding and the initial pre-disbudding concentration for each individual.

Study Course

Disbudding was performed in the early morning hours. Before the procedure, cameras and leg sensors were set in place. Local anesthesia and systemic analgesia were administered 20 minutes before disbudding. The same individuals were utilized throughout the study to avoid confounding variables performed due to animal restraint, blood sampling and administration of local anesthesia and pain relief. Moreover, if difficulty with restraint arose at any point, blood was not sampled from the animal.

Statistical Analysis

The statistical analysis for variability was carried out using a one-way ANOVA test and accompanied by a Toki-Kramer test to determine the difference between the groups (indicate by different letters; a, b or c). All tests, including calculation of averages and standard errors, were carried out using Jump Software (Windows, Macintosh, Utah, United States). Comparisons between the means were made using a student t-test at a significance level of $P \leq 0.05$. All Data are presented as the mean±standard error.

RESULTS

Physiological effects of disbudding among treatment groups

Effect of pain relief methods before disbudding on the blood cortisol levels:

In the first hour post-disbudding, the older group treated with lidocaine and NSAIDs showed minor increased changes in the cortisol level compared to the older control group, and the same cortisol level of changes were present in the naïve group (Figure 1). Additionally, the cortisol change of the older calves receiving local anesthetics was statistically significantly lower ($P=0.05$) than the older control group. Four hours post-disbudding there was no significant difference between the older treatment groups ($P=0.95$). Eight hours following the procedure the change in cortisol levels in the older calves differed only between naïve and o-lid ($P=0.042$) and naïve vs lid +NSAID ($P=0.041$) groups.

In the first hour post-disbudding, cortisol levels in the young group that received lidocaine or lidocaine+NSAID's were lower but did not differ from cortisol levels of their control calves ($P=0.73$). No statistical differences were found also between y-control and o-lid+ NSAID's calves ($P=0.52$). Four and eight hours post-disbudding no significant differences were found between the groups (Figure 2).

Behavioral effects of disbudding

Effect of pain relief methods before disbudding on the intensity of calves' head shakings

Intensity of head shaking in the older calves:

Within the first hour, the older control group (o-control) had a significant elevation of head shakings compare with the other groups, followed by remission in the successive hours. The older group treated with lidocaine (o-lid) showed in the first hour fewer head shakings than the control group. However, in the following hours, there was an increase in head-shaking reaching the highest intensity 4 hours post-disbudding. At that point, the head-shaking intensity of that group was significantly higher than the rest of the groups (Figure 3; *$P<0.001$*). The older group receiving NSAIDs

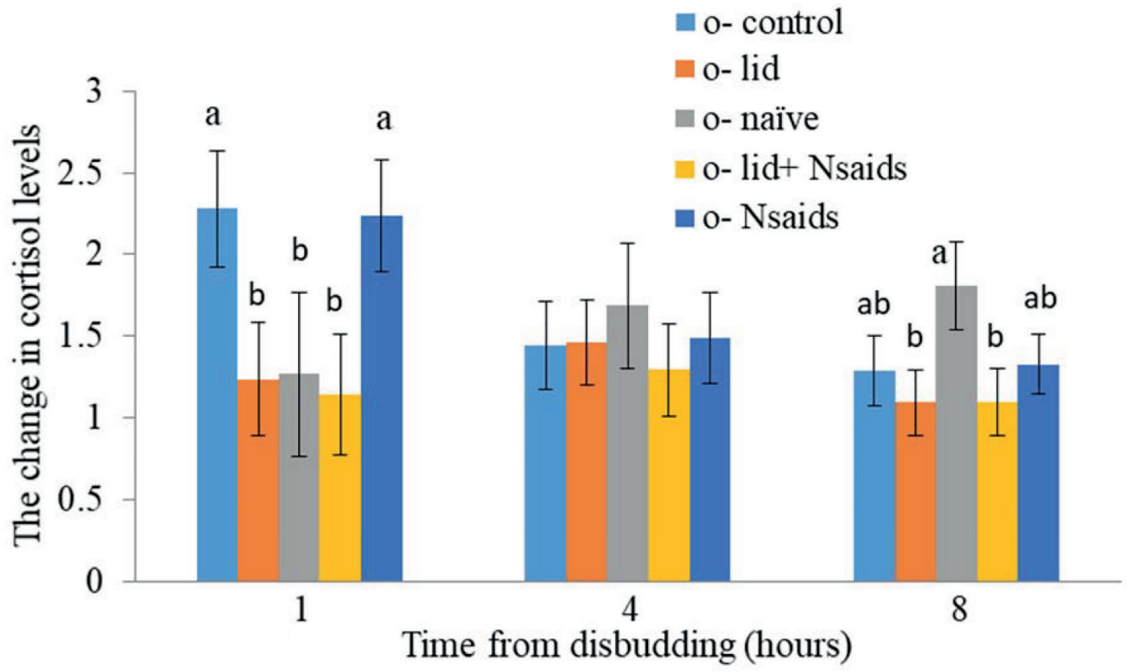

Figure 1. Effect of pain relief methods before disbudding on the change in cortisol blood levels in the older calf groups: Older control group (o-control), the older group treated with lidocaine (o-lid), older naïve group (o-naïve), the older group treated with lidocaine and NSAIDs (o-lid+Nsaids), the older group receiving NSAIDs only (o-NSAIDs).

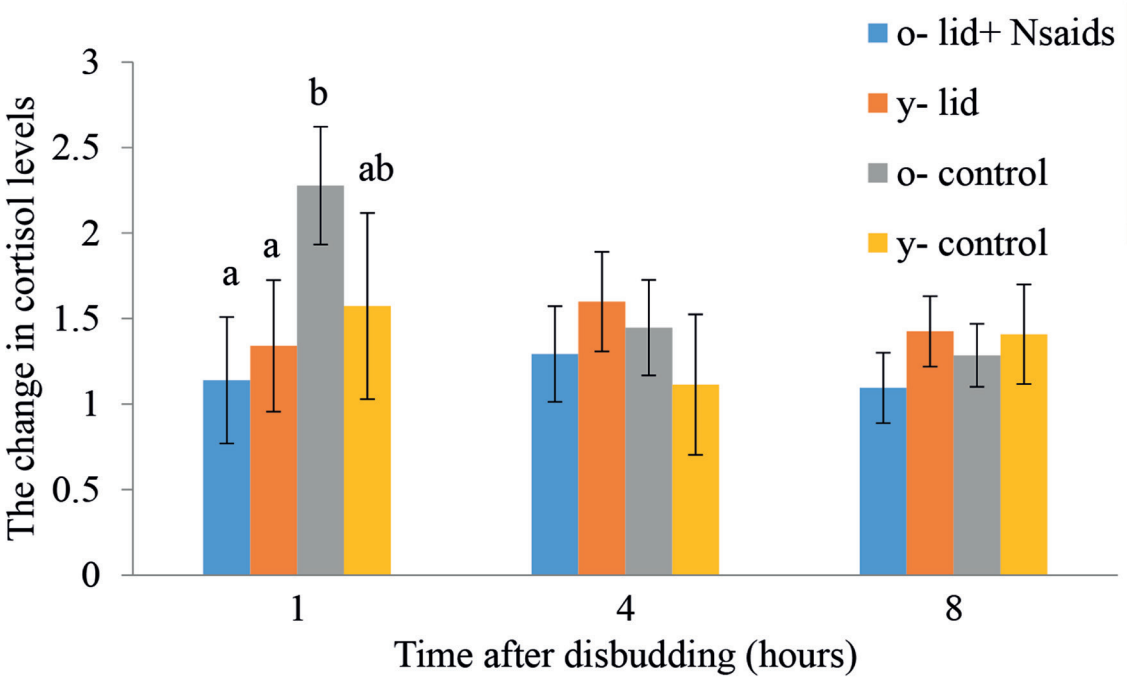

Figure 2. The change in cortisol blood levels post-disbudding between all groups: older control calves (o-control), Young control calves (y-control), the young group receiving lidocaine (y-lid), older calves receiving lidocaine and NSAIDs (o-lid+ NSAID's). Levels not connected by same letter were significantly different ($P<0.05$).

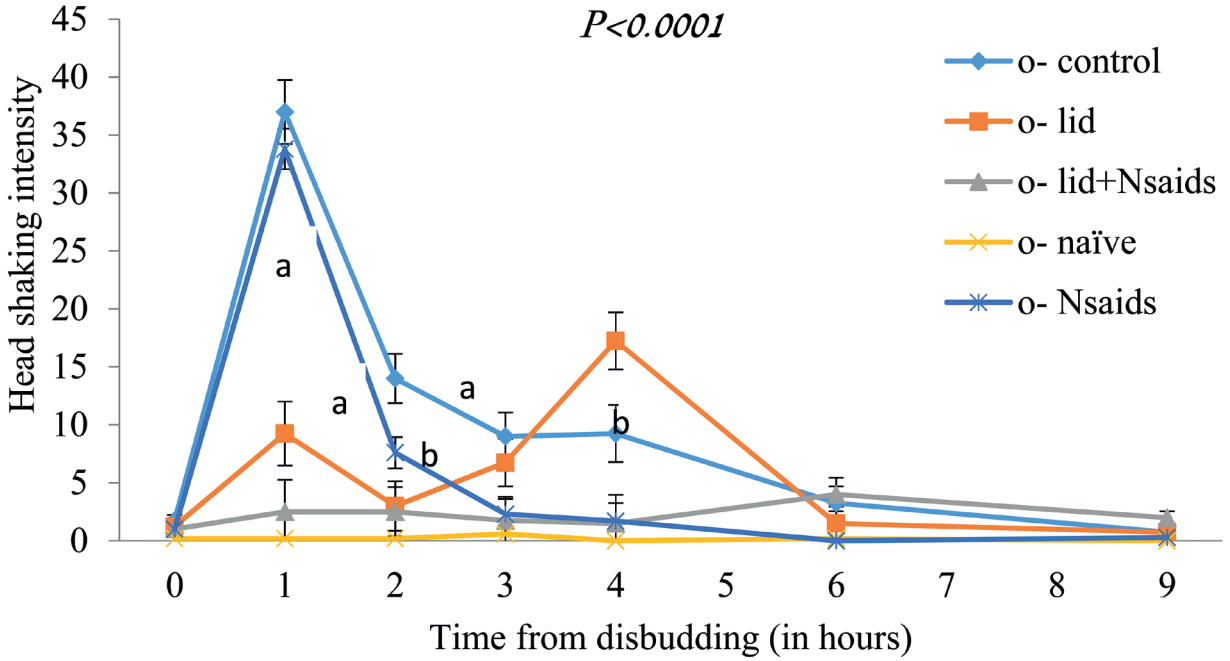

Figure 3. Effect of pain relief methods before disbudding on the intensity of head shakings in the older calves' groups: older control group (o-control), the older group treated with lidocaine (o-lid), older naïve group (o-naïve), the older group treated with lidocaine and NSAIDs (o-lid+Nsaids), the older group receiving NSAIDs only (o-NSAIDs). Levels not connected by same letter are significantly different. **(***P<0.001***)**

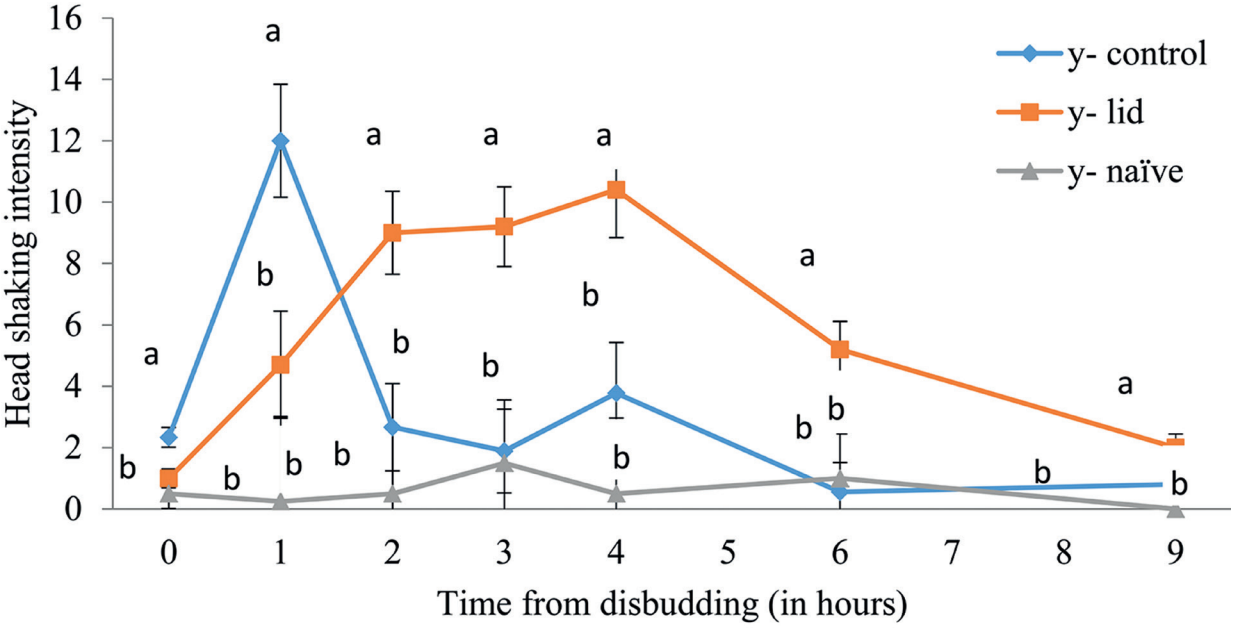

Figure 4. Effect of pain relief methods before disbudding on the intensity of head shakings in the younger calves' groups: younger group (y-control), the younger group treated with lidocaine (y-lid), younger naive group (y-naïve). The intensity of head-shaking was tested by counting the numbers of head shakings recorded by video, every hour following disbudding. Levels not connected by same letter are significantly different. *P<0.001*

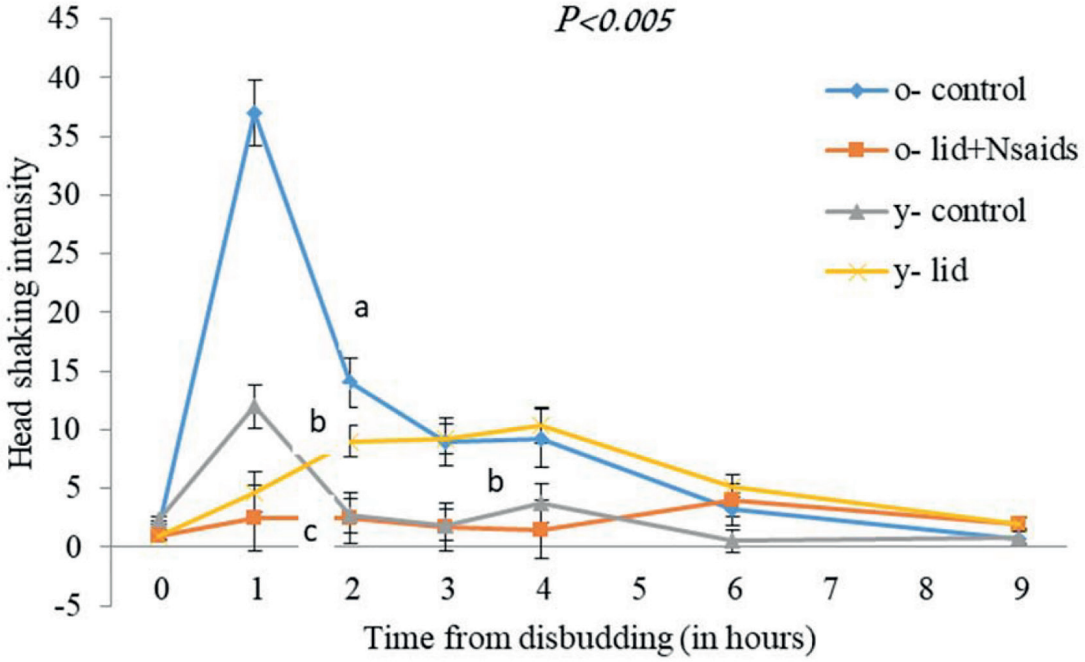

Figure 5. Effect of pain relief methods before disbudding on the intensity of head shakings in all calves' groups: younger group (y-control), the younger group treated with lidocaine (y-lid), older control calves (o-control), older calves receiving lidocaine and NSAIDs (o-lid+Nsaids). The intensity of head-shaking was tested by counting the numbers of head shakings recorded by video, every hour following disbudding. Levels not connected by same letter are significantly different. (*P<0.05)*

had a similar pattern as the older controls during the first-hour shaking peaked. In the two, three and four hours after disbudding, there was a significant decrease in head shaking comparing with the older control group. Six and nine hours post-procedure there were no differences for head shaking among treatments.

There were no significant differences in head-shaking intensity between the older group that received lidocaine + NSAIDs and the older naïve group throughout all measurements. Also, the older group receiving lidocaine + NSAIDs had the fewest head shakings off all other treatment groups, three and four hours post-disbudding.

Intensity of head shaking in the younger group

The disbudding caused a significant elevation in the number of head shakings in the control calves during the first hour (Figure 4; *P<0.001*). In the second hour, it decreased and continued to do so throughout all further time points. The younger group that received lidocaine pre-disbudding had a significantly lower number of head-shaking in the first hour compared to the control group. However, two hours post-procedure, head shaking intensity was higher than control and naïve groups. From two hours post-procedure and onwards, there were no differences between the naïve and control group.

Effect of pain relief methods before disbudding on the intensity of calves' head-scratching

The results of "head-scratching" which are presented in Figure 6 are similar to the data shown for "head-shakings". There were significant differences between o-control and y-control in the first hour after disbudding (Figure 6). From the comparison between o-lid+ NSAID's and y-lid, in the first-hour post-disbudding, there were no significant differences among groups. However, in the following hours, there was a significant increase in head-shaking intensity in the y-lid group, as opposed to the o-lid- NSAID's and y-control (Figure 6).

Effect of pain relief methods before disbudding on the calves' activity

In the first hour following disbudding, the mean number of steps presented by the young control calves was significantly higher than the rest of the treatment groups. Other than

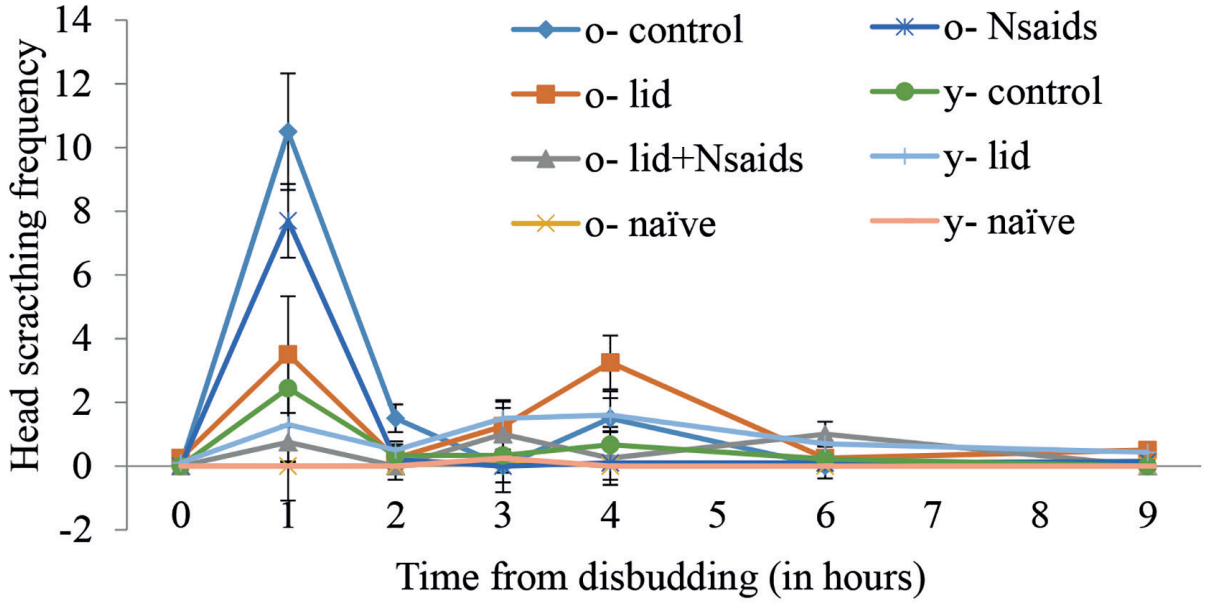

Figure 6. Effect of pain relief on head-scratching in all groups: older control calves (o-control), younger control calves (y-control), older calves which received lidocaine (o-lid), younger calves which received lidocaine (y-lid), older naïve calves (o-naïve), younger naïve calves (y-naïve), older calves which received NSAID's (o-NSAID's) and the older calves which received lidocaine and NSAIDs (o-lid + NSAID's).

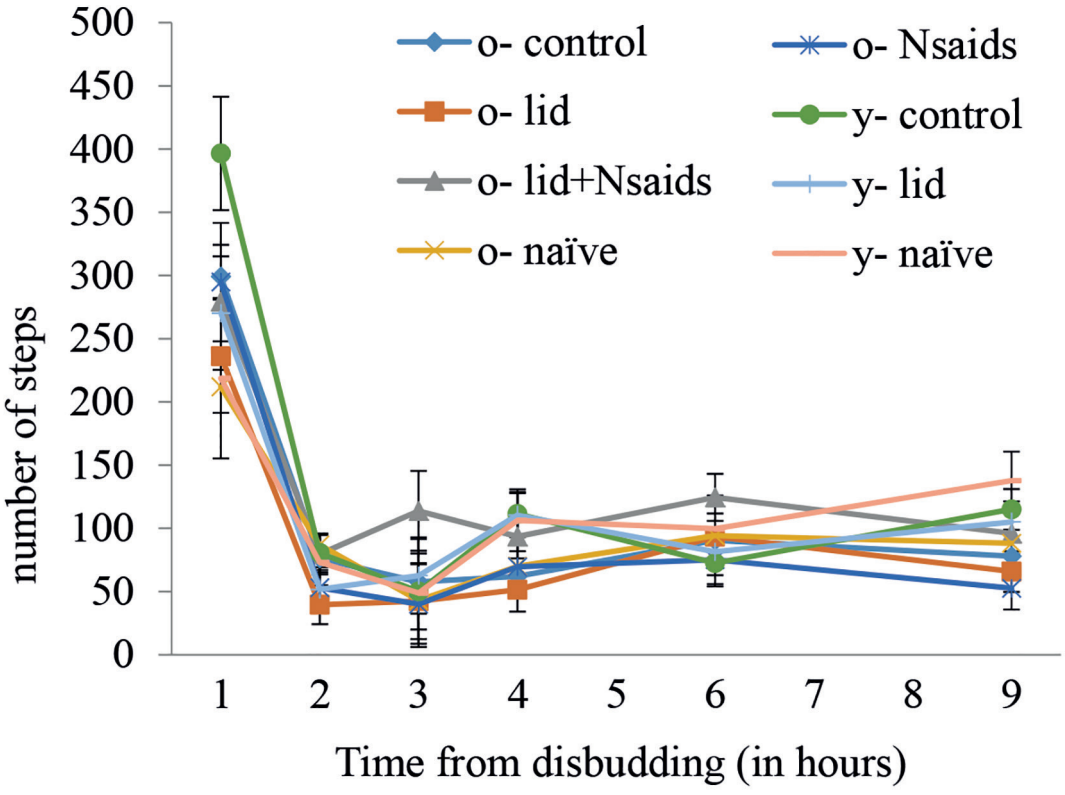

Figure 7. Effect of pain relief on calves' activity (expressed by the number of steps) in all groups: older control calves (o-control), younger control calves (y-control), older calves which received lidocaine (o-lid), younger calves which received lidocaine (y-lid), older naïve calves (o-naïve), younger naïve calves (y-naïve), older calves which received NSAID's (o-NSAIDs) and the older calves which received lidocaine and NSAIDs (o-lid+NSAID's).

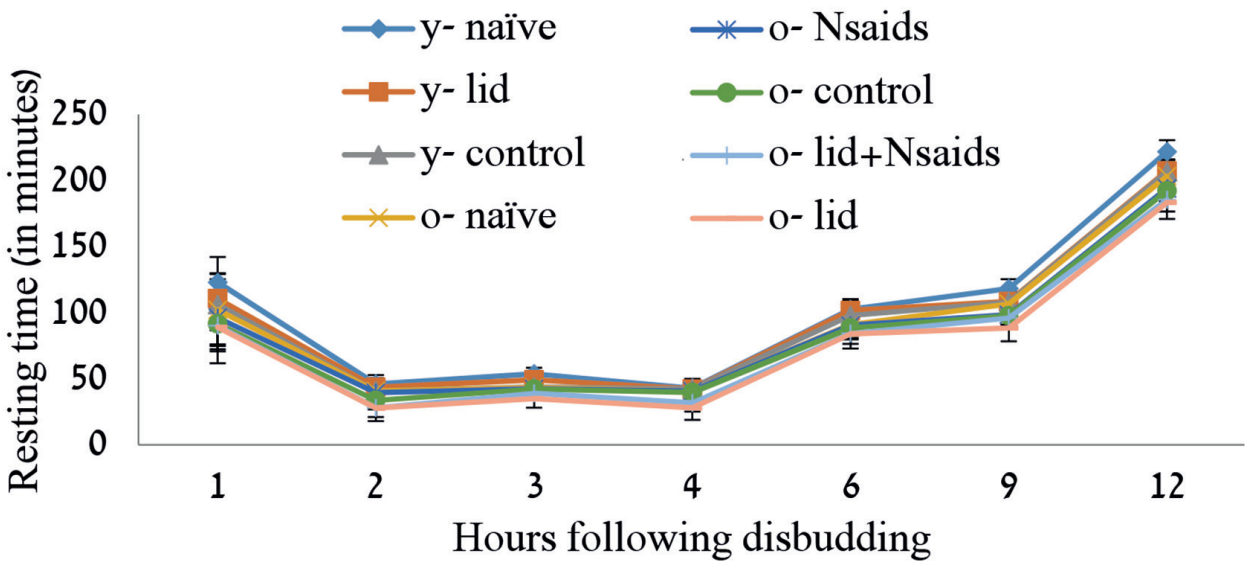

Figure 8. Effect of pain relief on calves' resting duration (expressed by minutes of recumbency) in all groups: older control calves (o-control), younger control calves (y-control), older calves which received lidocaine (o-lid), younger calves which received lidocaine (y-lid), older naïve calves (o-naïve), younger naïve calves (y-naïve), older calves which received NSAID's (o-NSAIDs) and the older calves which received lidocaine and NSAIDs (o-lid+NSAID's).

that, no further differences were noted among groups (Figure 7). Older control calves (o-control), younger control calves (y-control), older calves which received lidocaine (o-lid), younger calves which received lidocaine (y-lid), older naïve calves (o-naïve), younger naïve calves (y-naïve), older calves which received NSAIDs (o-NSAIDs) and the older calves which received lidocaine and NSAIDs (o-lid+NSAIDs). Statistical significance *(P<0.05)* is shown where the confidence interval is not overlapping between the treatments.

Effect of pain relief methods before disbudding on the calves' resting duration

A general increase in activity was demonstrated by the diminution in resting time derived from the pedometer data. However, no differences were found among treatment groups.

DISCUSSION

Cortisol levels

When comparing plasma cortisol levels during the first hour after disbudding calves treated with meloxicam (o-NSAIDs) and the control groups showed no significant differences. These result come in contradiction to those obtained in a study assessing pain and distress caused by hot iron disbudding with the treatment of meloxicam (22).This result may reflect the low relief that meloxicam has on caustic paste disbudding. At four and eight hours after disbudding, no significant differences in cortisol levels were shown between the meloxicam-treated group (o-NSAIDs) and the control group (o-control). These results do not collaborate with our hypothesis that calves treated with NSAIDs would show less pain and distress in the later hours after disbudding compared with control groups as it was shown four to six hours after disbudding (7, 24). These results indicate, again, the inferior influence that NSAIDs without local anesthetics on this type of pain.

Cortisol plasma concentration measured in the older group treated with lidocaine and meloxicam (o-Lid+NSAIDs) one hour after disbudding was significantly lower *(P=0.005)* than that measured in the control group (o-control), and was not significantly different than that measured in the naïve group (o-naïve). However, four and eight hours after disbudding there was no significant difference between any of the treatment groups (young and old) besides the naïve group (o-naïve), in which a significantly higher cortisol concentration was found compared to all older calf treatment groups as it was already shown in other disbudding research (24).

Significantly higher plasma cortisol levels (P=0.041) in

the o-naïve group eight hours after disbudding could be explained by cyclic peaks of the hormone, naturally occurring in the bloodstream (23, 24). Cortisol concentration in normal animals rises and falls irregularly throughout the day and is influenced by different mental and physical states such as stress, pain, hunger, etc. (25), however despite this, all farm parameters and time of day and procedure were kept the same during the experiment.

A possible explanation for high plasma cortisol eight hours after disbudding in the o-naïve group could be that pain and stress following disbudding affected normal cyclic hormonal behavior and the rise in plasma cortisol seen in naïve animals was a normal hormonal peak not occurring in treated animals due to the medical treatment.

To assess the optimal age for disbudding, we compared the treatment inducing the lowest plasma cortisol concentrations over time in the older age group (o-lid+NSAIDs) and the treatment inducing the lowest plasma cortisol concentration over time in the younger age group (y-lid). The two treatment groups were compared to the two control groups in the study. During the first hour after disbudding, plasma cortisol concentrations did not differ significantly between o-lid+NSAIDs, y-lid, y-control. However, cortisol concentration in the older control group was significantly higher than cortisol concentration in the y-lid (P=0.041) and 0-lid+NSAIDs groups (P=0.042), though not significantly different than the young control group. Therefore, it may be concluded that there may not be any advantage to the administration of local anesthetic if the procedure is performed at less than one week of age based on plasma cortisol levels and behavioral parameter's (head shaking& head scratching). Additionally, when examining plasma cortisol alone, there were no notable difference in performing the disbudding procedure between the two age groups in this study if no means of pain relief were administered. As previously noted, this conclusion was supported by a study concluding that histological examinations of the horn bud and the adjacent area in calves from birth until 4 months of age did not show apparent differences in the density of cutaneous innervation (26). However, this result differs from another study examining the pain reaction due to castration in calves, in which researchers showed significantly lower plasma cortisol levels in calves under six days of age compared with older calves. Additionally, the study showed that plasma cortisol levels gradually rise as calves grow older if no means of pain relief is administered (27). The incongruence of these results with the results of this current study could be due to a possible change in cutaneous innervation of the scrotum, as calves grow older (28).

Behavior parameters

Observing head shaking, in the older-control group, showed in our results a sharp rise in the frequency of head shaking seen during the first hour after disbudding, with an immediate fall soon after and during the following hours of the study. This trend was consistent with a similar study examining head shaking after disbudding with caustic paste with no administration of pain-relieving drugs (21). In the o-lid group, a significantly *(P<0.001*) lower amount of head-shaking was noted compared to the o-control group during the first hour after disbudding. However, during the following hours of the study, an increase in head shaking was seen in this treatment group, reaching a peak at four hours after disbudding, showing a significantly higher *(P<0.001*) amount of head-shaking than noted in any other treatment group of older calves. This finding is consistent with our hypothesis regarding the medical impact of local anesthesia and coincides with similar studies showing a significant effect of local anesthesia on signs of pain and distress during disbudding, with a significant rise in pain-related behavior compared to control groups in the hours following the procedure (8). In the o-NSAID's group, similarly to the results obtained in plasma cortisol levels, a sharp rise in head shaking was seen during the first hour after disbudding, peaking at a level not significantly different from that measured in the o-control group. However, during two, three, and four hours after disbudding, significantly *(P=0.05*) lower occurrences of head-shaking was measured compared to the o-control group. These results do not coincide with those obtained in plasma cortisol concentrations measurements.

The results described regarding the amount of head shaking, support our hypothesis regarding the late effects of NSAIDs on lowering pain-related behavior without affecting immediate pain-associated behavior. Researchers have shown a drop in pain-associated behavior approximately an hour after disbudding with an effect lasting up to six hours in calves disbudded with the administration of NSAID's (7).

No significant differences was noted between the o-naïve and the o-lid+NDSAID's groups in the frequency of head-shaking during all hours. Additionally, in the o-lid+NSAID's

group, the amount of head-shaking during one, three, and four hours post disbudding was significantly *(P=0.05*). lower than seen in both control groups and all groups receiving any means of pain relief (i.e. o-control, o-lid, o-NSAIDs). It is generally accepted that preventing pain is the best method of pain control (29). This data supports our hypothesis that the optimal treatment for reducing signs of pain and distress following disbudding is the administration of a combination of a local anesthetic to alleviate the pain during and shortly after disbudding and NSAIDs for pain relief and reduction of inflammation during the hours following, as described in a previous work (23).

To determine the optimal treatment for disbudding up to one week of age, we compared all treatments of the younger age group. In the y-lid group, a significantly lower amount of head-shaking was noted compared with the y-control group, and no significant difference was measured between this group and the y-naïve group during the first hour after disbudding. At two, three, and four hours after disbudding, significantly more head-shaking was seen in the y-lid group than in any other treatment groups of the younger age group, similarly to the results obtained in the older age group (Figure 5) . These results support our hypothesis regarding treatment with local anesthetic alone.

To determine the optimal age for disbudding the treatments producing the lowest incidences of head-shaking throughout all hours of the study in each age group were compared (y-lid and o-lid+NSAIDs). Additionally, we compared the control treatments of both age groups (y-control and o-control).

During one, two, three, and four hours after disbudding a significantly higher amount of head-shaking was measured in the o-control group compared to the y-control group (Figure 5). These results differ from the plasma cortisol concentration results (Figure 1 & 2). This difference may be explained by a study in which researchers found that cortisol responses were of a rather short duration compared to behavioral responses (30). This result may indicate that future investigations on pain in animals should include pain-related behavior as a high priority parameter (31).

Comparison of the o-lid+NSAIDs treatment and the y-lid treatment to control groups of both ages showed similar results to changes in plasma cortisol levels during the first hour, indicating that the amount of head-shaking was significantly lower in these treatment groups compared to control treatments of both age groups (y-control and o-control). However, during two, three and four hours after disbudding, the amount of head-shaking measured in the y-lid group was significantly higher than measured in the y-control and o-lid+NSAIDs groups, and not significantly different than seen in the o-control group.

Regarding head-scratching, comparison from different treatment groups in this category of pain-related behavior showed similar results to those shown in head shaking and therefore will not be further elaborated upon.

Pedometers parameters

The pedometers did not contribute in a major manner to the welfare understanding for this research. Therefore, it is difficult to attribute these results to the study itself. A possible explanation could be the presence of confounding factors such as feeding or external activity that created excitement in all the calves at a regular hour. Another possibility is that the number of steps measured did not properly represent calf distress or pain, as the calves housed in confined areas that do not allow much room for walking.

CONCLUSIONS

In this study, we examined the effect of administration of systemic analgesia and local anesthetic on pain and distress associated with the disbudding procedure in dairy calves. Additionally, we wished to determine if there was a preferable age for disbudding, under commonly practiced Israeli dairy farm husbandry. The pain elicited was evaluate by two main parameters: physiological and behavioral.

These results led to the conclusion that the most favorable treatment when evaluating all parameters in this study is the o-lid+NSAIDs, indicating that disbudding should preferably be performed between the age of 8-14 days old with the administration of a combination of local anesthesia and NSAIDs drugs. However, if one decides to disbud calves at a younger age, there is no proven advantage to the administration of local anesthesia and more stress and pain-related behavior was exhibited over time by an animal disbudded with local anesthesia as opposed to those disbudded with no medical treatment.

In addition to the immediate pain caused to the animal, there may be long-term importance to the stress and pain caused at a young age. It was speculated that stress at a young

age may increase the chance of morbidity, and impair food intake and calf development (30). A link has also been found between animal welfare and productivity. When the welfare of a cow is impaired, there is a direct effect on fertility, milk yield, and the incidence of udder infections in later life (31, 32). Therefore, it is possible to examine whether there is an effect of stress at a young age on these indices, and the economic aspect must be examined throughout the years of calf rearing to understand the full interfacial implications of removing horn buds with and without local anesthesia and analgesics.

Results of this study show that there is justification for use of systemic analgesia and local anesthetic in the disbudding procedure. Therefore, we concluded that the suitable age for disbudding is between the age of 8-14 days as below the age of 7 days there is no labeled use for NSAIDs.

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