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**Research Article**

# Validation of Effect of Mechanical and Thermal Nociceptive Thresholds on Efficacy of Intravenous Regional and Modified Four Point Nerve Block Anesthesia to desensitize limbs of Cattle

**Samane Yavari***

*Graduated from Cattle Clinic, University of Veterinary Medicine Hannover Foundation, Hannover, Germany*

***Corresponding Author**: Samane Yavari, Graduated from Cattle Clinic, University of Veterinary Medicine Hannover Foundation, Hannover, Germany. **E-mail:** DR81YAVARIS@GMAIL.COM

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## Abstract

Mechanical and thermal nociceptive thresholds have been demandable to measure the onset as well as duration of limb local anesthesia. In our study, two limb local anesthesia methods of the most routine and the fastest recognized well-known techniques of intravenous regional analgesia and another less administered technique of four-point nerve blocks were examined. The aim was considering the application of heat, and mechanical nociceptive thresholds to evaluate the quantity (onset) and quality (duration) of two local anesthesia methods. For that aim, two examinations were designed to evaluate application of thermal and mechanical nociceptive thresholds at the hind limb of dairy cows by their administration before and after local anesthesia, heat and mechanical force and pin pricks. Full onset of two mentioned local analgesia techniques at seven different sites of the hind limb (six sites for mechanical and one for thermal) were assessed. First study was designed to evaluate the efficacy of anesthesia methods in eight dairy cows and the second one to estimate the full onset of anesthesia in six dairy cows in a cross-over study design. There was not seen any significant difference between two anesthetized groups by mechanical force, pin pricks and thermal stimuli in both studies ($p > 0.05$). In our discussion and conclusion we did discuss the effect of thermal and mechanical nociceptive thresholds on pain assessment related to the locomotion system and we compared different threshold devices regarding their quality and eligibility to assess pain at the limb of dairy cows.

***Keywords:*** *Heat; Mechanical Threshold; IVRA; NBA; Hind Limb*

## Introduction

Lameness as a controversial disorder, is one of the most three reasons for premature culling, after infertility and mastitis in order to inadequate prevention measurements as well as treatment [13,38,41]. Due to lack of investigations evaluating the most appropriate local anesthesia methods and validation of the most routine techniques to assess pain during and after application of local analgesia, nowadays, examinations of different methods and means to find the most beneficial local anesthesia techniques has been demandable. Two local anesthesia methods of, intravenous regional anesthesia (IVRA) [1,13,29] and nerve blocks [48] are recommended for surgeries of bovine distal limb. For the first time, the comparison of the efficacy and onset of two local anesthetic methods consist of IVRA and modified four-point NBA by means of electrical nociceptive thresholds revealed the possibility of application as well as complete desensitization of distal hind limb in dairy cows using the modified four-point NBA and faster full onset of modified four-point NBA about ten minutes than IVRA [45]. Previously two nociceptive stimuli of mechanical and thermal nociceptive thresholds in human [25] and some species such as cow [14,21,30], horse [7,20,31], canine [15], feline [2,34,36] or even in rabbit [5] were studied. That is noticeable to mention, the electrical nociceptive thresholds administered to measure the onset and efficacy of local anesthesia of hind limb in dairy cows for the first time by Yavari and her colleagues [45]. There is no publication of such that investigations to study in bovine medicine, except usage of electrical thresholds to improve of meat tenderness of forage-finished beef [17]. Mechanical [21,42] and heat [14,30] nociceptive thresholds were previously examined in dairy cows. There are different heat stimulators to use in dairy cows for instance laser-based devices [30,40], wireless thermal thresholds [27,28] or radiant heat in horses [7]. Some examples of application of mechanical threshold can be pneumatically actuated blunt pin [27], pressure nociceptive threshold [10], the pneumatic actuator to drive a pin into the tissue [9] or a pneumatically operated piston pressing a blunt ended pin on the animals' body under the computer control [7]. Mechanical nociceptive stimulators are commonly used due to easiness to administer at the limb [8], while thermal nociceptive thresholds can be selected to measure pain in order to their reliability to measure the nociception by behavioral responses [14]. Therefore, the focus of this publication would be the consideration of effectiveness of thermal and mechanical thresholds application to measure pain at the hind limb of dairy cows. We consider the effect of those nociceptive thresholds regarding to the local anesthesia.

## Materials and Methods

The study was approved by Niedersächsisches Landesamt für Verbraucherschutz und Lebensmittelsicherheit under the research permit number (33.19-42502-04-1511970).

### Study 1

### Animals and study design

Eight non-lactating/pregnant and healthy German Holstein Friesian dairy cows in a cross- over design (2 x 4) were selected. The animals were divided into two groups. The animals of group 1 received 20 ml Procaine (Procasel 2%, Selectavet) under Intravenous Regional Anesthesia while, 10 - 15 ml Procaine per each injection sites was applied for our modified four point nerve block anesthesia in the cows of group 2. The study was performed in the duration of about 50 minutes before LR, 60 minutes in LR on surgical tipping table and afterwards 30 minutes following returning cows to standing position from LR. All cows were housed together in free stall before study. They received routine diet for healthy non-pregnant/non-milking dairy cows.

### Intravenous regional anesthesia

Intravenous Regional Anesthesia was administered using two different hind limb vein digitalis lateralis communis IV was used to inject 20 ml Procasel 2% after application of tourniquet. After lateral recumbency and fixing the legs with tipping table bandage, the right hind leg was washed with water and soap and cleaned carefully at the area of anesthesia's administration and afterwards this area was shaved. After drying the cleaned shaved anesthesia's area, alcohol and iodine were applied respectively to disinfect the planned areas. Four min before administration of Intravenous Regional Anesthesia, rubber tourniquet was fixed around the leg above the fetlock joint at metatarsal region and then IVRA was applied distal to the tourniquet. In cases which reaching the vein digitalis lateralis communis IV was not possible, digitalis dorsalis communis III was applied for intravenous regional anesthesia.

### Modified four point nerve block anesthesia

To desensitize the right hind leg in lateral recumbent dairy cows, in overall, 50 - 70 ml Procasel 2% was applied. The dosage of anesthetic was not cared in order to different cows' body weights and so on. In average, 62.2 ml Procasel 2% was used per each cow for this anesthesia method. For our modified 4-point NBA, the anesthesia's regions include of dorsal, lateral and medial sites of metatarsus were washed and cleaned. Thereafter, the areas of injections for 4 - point nerve block anesthesia, consisting of superficial and deep peroneal (fibular) nerves, as well as lateral and medial plantar nerves of the tibial nerve, were shaved carefully. Then, shaved and cleaned areas were scrubbed carefully with the piece of alcohol and then iodine. Afterwards, to desensitize the superficial fibular nerve, about 15 ml Procasel 2% with 21 gauge needle was injected directly beneath the skin subcutaneously at the dorsal hind leg about 5 cm proximally to the hock joint (Figure 1). Thereafter to desensitize the deep fibular nerve about 15 ml Procasel 2% using 21 gauge needle was injected carefully into the proximal region of the dorsal longitudinal groove (sulcus longitudinalis dorsalis) of the metatarsus (Figure 2) under careful injection due to accompany the dorsal metatarsal vein and artery with deep fibular nerve in the groove (Figure 3). Then, to desensitize the lateral plantar nerve, after finding superficial and deep flexor tendons, about 15 ml track of Procasel 2% using needle gauge 21 was injected in the middle of lateral metatarsus peri-neurally in the palpable groove dorsal to the superficial and deep flexor tendons (Figure 4). Finally to desensitize the medial plantar nerve, about 15 ml track of Procasel 2% using needle gauge 21 was injected in the middle of medial metatarsus pri-neurally dorsal to the superficial flexor tendon (Figure 5).

***Figure 1:*** *The point of scalpel shows superficial fibular nerves.*

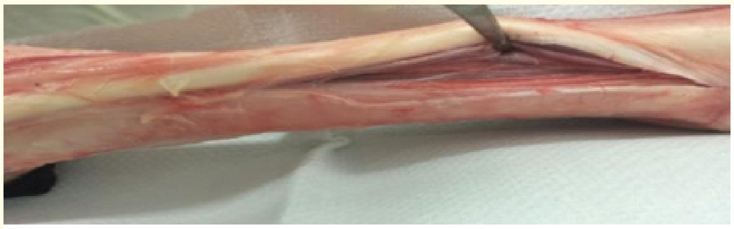

***Figure 2:*** *Dorso-medial aspect of metatarsus. Deep fibular nerve alongs with the dorsal metatarsal artery and vein in the groove.*

***Figure 3:*** *Cross dissection of metatarsus. Dorsal metatarsal vein, Deep fibular nerve and Dorsal metatarsal artery.*

***Figure 4:*** *The point of needle shows lateral plantar nerve.*

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***Figure 5:*** *The point of needle shows medial plantar nerve.*

### Mechanical nociceptive threshold

Mechanical measurements were done by using pin pricks as well as force in Newton. To measure mechanical nociceptive responses, pin pricks and force in Newton were administered in three different sites. First, lateral and medial sites of fetlock joint, second lateral and medial flexor tendon and third, lateral and medial heel bulb three times (t-10 min, t+15 min and t+30 min; t0 = anesthesia time). By this method, the pressure required to produce a response from the cattle was identified by movement of claw. The identification numbers for mechanical recording were 0 - 5 for pin pricks and 0 - 20 for force in Newton. Mechanical nociceptive thresholds were applied after finishing heat stimulation.

### Heat nociceptive threshold

Measuring thermal nociceptive threshold was done by using Wireless Thermal Threshold Testing System (WTT) (Topcat metrology Ltd) with the thermal cut-out at 55°C. The thermal threshold probe was attached to the shaved cleaned dorso-lateral coronary band of the right claw. The responses of heat nociception thresholds were recorded 10 minutes before anesthesia while 2 other nociception stimulations were administered 15 minutes and 30 minutes after anesthesia in the lateral recumbent cows. Movement of claw after application of thermal stimulation thorough increasing the heat nociception threshold was realized as a thermal nociceptive response and the temperature at which dairy cow responded to the stimuli was recorded as a thermal nociceptive threshold.

## Study 2

### Animals and study design

Six non-lactating/pregnant and healthy German Holstein Friesian dairy cows in a cross over design (2 x 3) were used under two treatments with either IVRA or NBA. The examined cows in study 2 were different from study 1. The animals were divided into two groups. The animals of group 1 received 20 ml Procaine (Procasel 2%, Selectavet) under Intravenous Regional Anesthesia while, in average 60 ml Procaine was applied for our modified 4 - point Nerve Block Anesthesia in the cows of group 2. The examination time schedule of study was around 20 minutes standing position after getting cows from the free stall, 40 minutes in lateral recumbency on surgical tipping table and finally 5 minutes after returning cows to standing position from lateral recumbency.

### Local anesthesia

The intravenous regional anesthesia and modified four point nerve block anesthesia were performed as study 1.

### Mechanical nociceptive threshold

All steps as study 1 mechanical nociceptive threshold responses were recorded using force tool in Newton and pin pricks at six different sites includes of lateral and medial heel bulb, dorsalis fetlock joints and flexor tendon 5 minutes before administration of anesthesia as well as 5, 7.5, 10, 12.5, 15 and 20 minutes after anesthesia application (t0 = anesthesia time).

### Statistical evaluations

Statistical evaluation was done by means of the Statistical Analysis System package (SAS version 9.3 for Windows, SAS institute Inc, Cary, NC, USA): ANOVA for repeated measurements (GLM) as well as analysis of variance and T-Tests as well as additionally Wilcoxon Test for non-parametric pin pricks noxious stimuli responses. The evaluated data were presented as Mean ± SD. The nociceptive threshold responses were assessed before and after anesthesia. As a repeated measurement, the level of significance was set at $P < 0.05$ for all parameters while for T-Test, α correction of p-values were performed. Therefore, the level of significance for T-Test of nociceptive threshold responses was set at $P < 0.01$.

## Results

### Study 1

The Mean ± SD scores regarding thermal and mechanical nociceptive thresholds have been presented in table 1.

| Time related to local anesthesia (minutes) Statistics | | | | | | | | | | |
|---|---|---|---|---|---|---|---|---|---|---|
| | | **t-10** | | **t15** | | **t35** | | | | |
| **Parameter** | **Group** | **Mean** | **SD** | **Mean** | **SD** | **Mean** | **SD** | **Group** | **Time** | **Group* time** |
| TTR (°C) | IVRA | 53.46 | 2.28 | 55 | | 55 | | 0.46 | **0.0008**$^{**}$ | 0.57 |
| | NBA | 52.45 | 3.01 | 55 | | 55 | | | | |
| Lat. Bulb MPR | IVRA | 12.62 | 3.99 | 20 | | 20 | | **0.032**$^{*}$ | **< .0001**$^{**}$ | **0.010**$^{*}$ |
| | NBA | 17.50 | 4.34 | 20.12 | 0.35 | 20 | | | | |
| Med. Bulb MPR | IVRA | 16.12 | 3.97 | 20 | | 20 | | 0.53 | **0.0011**$^{**}$ | 0.44 |
| | NBA | 17.81 | 4.30 | 19.75 | 0.70 | 20 | | | | |
| Lat. Bulb MNR | IVRA | 3.62 | 0.91 | 0 | | 0 | | 0.12 | **< .0001**$^{**}$ | 0.028 |
| | NBA | 2.37 | 1.59 | 0.12 | 0.35 | 0 | | | | |
| Med. Bulb MNR | IVRA | 2.87 | 1.45 | 0 | | 0 | | 0.86 | **< .0001**$^{**}$ | 0.97 |
| | NBA | 3.00 | 1.41 | 0 | | 0 | | | | |
| Lat. Ten MPR | IVRA | 18.62 | 2.32 | 20 | | 20 | | 0.11 | 0.07 | 0.07 |
| | NBA | 20 | | 20 | | 20 | | | | |
| Med. Ten MPR | IVRA | 18.62 | 2.53 | 20 | | 20 | | 0.09 | 0.059 | 0.059 |
| | NBA | 20 | 0.53 | 20 | | 20 | | | | |
| Lat. Ten MNR | IVRA | 1.62 | 1.18 | 0 | | 0 | | 0.54 | **<.0001**$^{**}$ | 0.68 |
| | NBA | 2.12 | 1.95 | 0 | | 0 | | | | |
| Med. Ten MNR | IVRA | 1.87 | 0.99 | 0 | | 0 | | 0.17 | **<.0001**$^{**}$ | 0.14 |
| | NBA | 1.00 | 1.41 | 0 | | 0 | | | | |
| Lat. Dors MPR | IVRA | 18.87 | 2.10 | 20 | | 20 | | 0.11 | 0.18 | 0.08 |
| | NBA | 20.12 | 0.35 | 20 | | 20 | | | | |

***Table 1:*** *Mean ± SD scores of Thermal Threshold Response (TTR), Electrical Threshold Response (ETR), Mechanical Pressure Response at Lateral Heel Bulb (Lat. Bulb MPR), Mechanical Pressure Response at Medial Heel Bulb (Med. Bulb MPR), Mechanical Needle Response at Lateral Heel Bulb (Lat. Bulb MNR), Mechanical Needle Response at Medial Heel Bulb (Med. Bulb MNR), Mechanical Pressure Response at Lateral Flexor Tendon (Lat. Ten MPR), Mechanical Pressure Response at Medial Flexor Tendon (Med. Ten MPR), Mechanical Needle Response at Lateral Flexor Tendon (Lat. Ten MNR), Mechanical Needle Response at Medial Flexor Tendon (Med. Ten MPR), Mechanical Pressure Response at Lateral Dorsal Fetlock Joint (Lat. Dors MPR), Mechanical Pressure Response at Medial Dorsal Fetlock Joint (Med. Dors MPR), Mechanical Needle Response at Lateral Dorsal Fetlock Joint (Lat. Dors MNR), Mechanical Needle Response at Medial Dorsal Fetlock Joint (Med. Dors MNR) in 8 cows that were treated (t0 = anesthesia time) with either Intravenous Regional Anesthesia (IVRA) or Nerve Block Anesthesia (NBA) in lateral recumbency. To measure Mechanical pressure Response (MPR), Force in Newton and to record Mechanical Needle Response (MNR), Needle Pricks were used.*

*G: Treatment effect; T: Time effect; T×G: Time × Treatment effect; IVRA: Intravenous Regional Anesthesia; NBA: Nerve Block Anesthesia; LR: Lateral Recumbency; TTR: Thermal Threshold Response; Lat. Bulb MPR: Mechanical Pressure Response at Lateral Heel Bulb; Lat. Bulb MNR: Mechanical Needle Response at Lateral Heel Bulb; Lat. Ten MPR: Mechanical Pressure Response at Lateral Flexor Tendon; Lat. Ten MNR: Mechanical Needle Response at Lateral Flexor Tendon; Lat. Dors MPR: Mechanical Pressure Response at Lateral Dorsal Fetlock Joint; Lat. Dors MNR: Mechanical Needle Response at Lateral Dorsal Fetlock Joint; Med. Bulb MPR: Mechanical Pressure Response at Medial Heel Bulb; Med. Bulb MNR: Mechanical Needle Response at Medial Heel Bulb; Med. Ten MPR: Mechanical Needle Response at Medial Flexor Tendon; Med. Ten MPR: Mechanical Pressure Response at Medial Flexor Tendon; Med. Dors MPR: Mechanical Pressure Response at Medial Dorsal Fetlock Joint; Med. Dors MNR: Mechanical Needle Response at Medial Dorsal Fetlock Joint.*

α correction set to $P < 0.016$ as a significant group difference between two treatment groups per each examination.

*Bold values differ significantly from baselines.*

$^{*}P < 0.05$; $^{**}P < 0.01$.

## Nociceptive threshold responses

Assessed results showed no significant group difference regarding type of anesthesia following application of thermal nociceptive thresholds while only the significant effect of time ($P = 0.0008$) related to thermal nociceptive threshold responses was observed. Regarding mechanical pressure and pin pricks nociceptive thresholds, any significant group difference after anesthesia application was not observed at any measured sites, except significant group effect of mechanical force threshold at lateral heel bulb ($P = 0.032$) which is related to control group before anesthesia. However, remarkable time effect ($P < 0.05$) was seen at lateral and medial heel bulb for mechanical pressure and pin prick thresholds as well as flexor tendons at the lateral and medial digit as well as dorsal surface of the fetlock joint for mechanical pin pricks responses. Moreover, significant interaction between time and group was observed related to pressure and pin pricks threshold responses at lateral heel bulb as well as pin prick threshold response at medio-dorsal surface of the fetlock joint ($P < 0.05$).

## Mechanical nociceptive threshold responses

No significant group difference was seen after anesthesia with either IVRA or NBA at all of examined sites consist of lateral and medial coronary band, flexor tendon and dorsal aspect of fetlock joint ($P > 0.05$). All cows had a maximum mechanical pressure nociceptive threshold response 5 minutes following anesthesia while all of examined cows had no reaction to pin pricks 10 minutes after anesthesia with either IVRA or NBA at lateral and medial dorsal aspect of fetlock joint and flexor tendon. However, all examined cows showed no response to mechanical pressure nociceptive thresholds 7,5 minutes as well as pin pricks 10 minutes after anesthesia application at lateral and medial heel bulb. Regarding mechanical pressure nociceptive threshold responses any significant group and interaction between group and time (group*time) was not seen at any measured site ($P > 0.05$). However, the evaluated mechanical pressure nociceptive responses revealed significant time effect at medial dorsal surface of fetlock joint ($P = 0.028$), medial surface of flexor tendon ($P < 0.0001$) and lateral and medial heel bulb ($P < 0.0001$ and $P = 0.0005$) respectively. Furthermore, related to mechanical pin pricks responses, significant time effect was observed at all six measured sites consist of lateral and medial dorsal aspect of fetlock joint, flexor tendon and heel bulb ($P < 0.0001$).

## Heat nociceptive threshold responses

According to the results, the mean heat nociceptive threshold responses in both treated groups with either nerve block anesthesia or intravenous regional anesthesia was the same after treatment at t+15 min as well as t+35 min. However, the effect of time was significant (Time effect: $P = 0.0008$) even though there was not seen any effect of treatment and its interaction with time (Group effect: $P = 0.46$; Time*Group effect: $P = 0.57$). Therefore, in other words, regarding heat threshold responses, the interaction between period and anesthesia as well as treatment with either IVRA or our modified NBA had no effect on pain ($P > 0.01$).

## Study 2

The Mean ± SD scores of mechanical pin pricks and force in newton have been shown in table 2.

## Mechanical nociceptive threshold responses

The mean mechanical pressure nociceptive responses in our modified 4-point NBA group was higher than IVRA group at lateral heel bulb while mean mechanical pressure nociceptive responses in nerve block anesthetized group was at the exactly same level with intravenous regional treated cows at medial heel bulb as well as lateral and medial flexor tendon and lateral and medial dorsal aspect of fetlock joint fifteen minutes after anesthesia. However, mean mechanical pin pricks threshold responses in nerve block anaesthetized groups was greater than intravenous regional anaesthetized groups at lateral heel bulb while mean mechanical pin pricks threshold responses in intravenous regional treated cows was higher than nerve block anesthetized groups at medial dorsal aspect of fetlock joint with the exactly same level at other examined sites fifteen minutes following anesthesia with either intravenous regional or nerve block anesthesia method. The mean mechanical pressure and pin pricks threshold responses in nerve block anaesthetized groups and intravenous regional treated ones had an exactly same level at lateral and medial heel bulb, lateral and medial flexor tendon as well as lateral and medial

| Time related to local anesthesia (minutes) | | | | | | | | | | | | | | | | | | |
|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|
| | | t-5 | | t5 | | T7.5 | | T10 | | T12.5 | | T15 | | T20 | | | | |
| **Parameter** | **Group** | **Mean** | **SD** | **Mean** | **SD** | **Mean** | **SD** | **Mean** | **SD** | **Mean** | **SD** | **Mean** | **SD** | **Mean** | **SD** | **Group** | **Time** | **Group* time** |
| Lat. Bulb MPR | IVRA | 11.66 | 5.71 | 19.16 | 2.04 | 20 | | 20 | | 20 | | 20 | | 20 | | 0.63 | < .0001** | 0.99 |
| | NBA | 12.50 | 4.92 | 20 | | 20 | | 20 | | 20 | | 20 | | 20 | | | | |
| Med. Bulb MPR | IVRA | 16.66 | 5.12 | 19.16 | 2.04 | 20 | | 20 | | 20 | | 20 | | 20 | | 0.85 | 0.0005** | 0.99 |
| | NBA | 16.50 | 5.43 | 20 | | 20 | | 20 | | 20 | | 20 | | 20 | | | | |
| Lat. Bulb MNR | IVRA | 2.50 | 1.97 | 0.66 | 0.81 | 0 | | 0 | | 0 | | 0 | | 0 | | 0.58 | < .0001** | 0.85 |
| | NBA | 2.50 | 1.37 | 0 | | 0 | | 0 | | 0 | | 0 | | 0 | | | | |
| Med. Bulb MNR | IVRA | 2.50 | 1.64 | 0.5 | 0.54 | 0.08 | 0.2 | 0 | | 0 | | 0 | | 0 | | 0.58 | < .0001** | 0.88 |
| | NBA | 2.66 | 0.81 | 0 | | 0 | | 0 | | 0 | | 0 | | 0 | | | | |
| Lat. Ten MPR | IVRA | 20 | | 20 | | 20 | | 20 | | 20 | | 20 | | 20 | | 0.34 | 0.43 | 0.43 |
| | NBA | 19.33 | 1.63 | 20 | | 20 | | 20 | | 20 | | 20 | | 20 | | | | |
| Med. Ten MPR | IVRA | 16.83 | 3.76 | 20 | | 20 | | 20 | | 20 | | 20 | | 20 | | 0.34 | <.0001** | 0.99 |
| | NBA | 16.33 | 5.16 | 20 | | 20 | | 20 | | 20 | | 20 | | 20 | | | | |
| Lat. Ten MNR | IVRA | 1 | 1.26 | 0.16 | 0.4 | 0.08 | 0.2 | 0 | | 0 | | 0 | | 0 | | 0.75 | <.0001** | 0.99 |
| | NBA | 1 | 1.09 | 0 | | 0 | | 0 | | 0 | | 0 | | 0 | | | | |
| Med. Ten MNR | IVRA | 1.83 | 1.16 | 0.75 | 1.25 | 0.25 | 0.41 | 0.25 | 0.41 | 0.08 | 0.02 | 0.08 | 0.2 | 0 | | 0.1 | <.0001** | 0.21 |
| | NBA | 1 | 0.89 | 0 | | 0 | | 0 | | 0 | | 0 | | 0 | | | | |
| Lat. Dors MPR | IVRA | 19.83 | 0.4 | 20 | | 20 | | 20 | | 20 | | 20 | | 20 | | 0.37 | 0.34 | 0.53 |
| | NBA | 17.83 | 5.3 | 20 | | 20 | | 0 | | 20 | | 20 | | 20 | | | | |
| Med. Dors MPR | IVRA | 19.83 | 0.4 | 20 | | 20 | | 20 | | 20 | | 20 | | 20 | | 0.18 | 0.028* | 0.08 |
| | NBA | 17.33 | 4.32 | 20 | | 20 | | 20 | | 20 | | 20 | | 20 | | | | |
| Lat. Dors MNR | IVRA | 1.25 | 1.17 | 0 | | 0 | | 0 | | 0 | | 0 | | 0 | | 0.68 | <.0001** | 0.98 |
| | NBA | 1 | 0.89 | 0 | | 0 | | 0 | | 0 | | 0 | | 0 | | | | |
| Med. Dors MNR | IVRA | 0.91 | 1.02 | 0.08 | 0.2 | 0 | | 0 | | 0 | | 0 | | 0 | | 0.68 | <.0001** | 0.54 |
| | NBA | 0.5 | 0.54 | 0.16 | 0.4 | 0.08 | 0.2 | 0 | | 0 | | 0 | | 0 | | | | |

***Table 2:*** *Mean ± SD scores of Mechanical Pressure Response at Lateral Heel Bulb (Lat. Bulb MPR), Mechanical Pressure Response at Medial Heel Bulb (Med. Bulb MPR), Mechanical Needle Response at Lateral Heel Bulb (Lat. Bulb MNR), Mechanical Needle Response at Medial Heel Bulbl (Med. Bulb MNR), Mechanical Pressure Response at Lateral Dorsal Fetlock Joint (Lat. Dors MPR), Mechanical Pressure Response at Medial Dorsal Fetlock Joint (Med. Dors MPR), Mechanical Needle Response at Lateral Dorsal Fetlock Joint (Lat. Dors MNR), Mechanical Needle Response at Medial Dorsal Fetlock Joint (Med. Dors MNR), Mechanical Pressure Response at Lateral Flexor Tendon (Lat. Ten MPR), Mechanical Pressure Response at Medial Flexor Tendon (Med. Ten MPR), Mechanical Needle Response at Lateral Flexor Tendon (Lat. Ten MNR) and Mechanical Needle Response at Medial Flexor Tendon (Med. Ten MNR) in 6 cows that were treated (t0 = anesthesia time) with either Intravenous Regional Anesthesia (IVRA) or Nerve Block Anesthesia (NBA) in lateral recumbent dairy cows.*

dorsal aspect of fetlock joint at t+35 min, 35 minutes after anesthesia. Regarding mechanical pressure nociceptive responses, evaluation of recorded results revealed no significant group difference related to type of anesthesia at lateral and medial heel bulb (respectively P = 0.35 and P = 0.35). Furthermore, the mechanical pressure and pin pricks responses at all of the measured sites in both treatment methods were same and no difference was seen between two anesthetized groups 30 minutes after anesthesia. The mechanical pressure nociceptive threshold responses at lateral bulb revealed a significant effect of time as well as its interaction with anesthesia method (Time effect: P < 0.0001; Time*Group effect: P = 0.010) while the effect of anesthesia type was not significant (Group effect: P = 0.03). At the medial bulb only the significant effect of time (Time effect: P = 0.0011) while treatment method as well as interaction between time and type of anesthesia had no effect on pain (Group effect: P = 0.53; Time*Group effect: P = 0.44), at the medial tendon showed no significant effect of time, group as well as interaction between time and group (Group effect: P = 0.098; Time effect: P = 0.059; Time*Group effect: P = 0.059), at dorso-lateral surface of metatarsus showed that there was not any significant effect of time, treatment as well as time and treatment together (Time effect: P = 0.18; Group effect: P = 0.11; Group*Time effect: P = 0.08), also, at its medial site revealed no effect of time, anesthesia type and their interaction with each other (Time effect: P = 0.09; Group effect: P = 0.78; Time*Group effect: P = 0.92) and at Lat. Ten revealed no significant effect of treatment and time and their interaction with each other (Time effect: P = 0.07; Group effect: P = 0.11; Group*Time effect: P = 0.07), while the mechanical needle pricks nociceptive threshold responses at Lat. Bulb demonstrated only significant time and its interaction with group (Time effect: P < 0.0001; Time*Group effect: P = 0.028) while there was not seen any significant effect of anesthesia (Group effect: P = 0.12), at Med. Bulb showed the significant effect of time (Time effect: P < 0.0001) while any effect of treatment method and its interaction and time was not seen (Group effect: P = 0.86; Time*Group effect: P = 0.97), at Lat. Dors. showed although time had a significant effect on pain (Time effect: P = 0.0004) any significant effect of treatment and its interaction with time was not seen (Group effect: P = 0.1; Group*Time effect: P = 0.08) and at Lat. Ten revealed that only the effect of time (P < 0.0001) while the impact of treatment and its interaction and time was not significant (Group effect: P = 0.5; Group*Time effect: P = 0.6), at Med. Ten again revealed the significant effect of time (Time effect: P < 0.0001) and no significant impact of anesthesia and treatment with time together (Group effect: 0.17; Time*Group effect: P = 0.14), at Med. Dors clearly revealed no significant effect of treatment as well as time interaction with treatment (Group effect: P = 0.06; Time*Group effect: P = 0.03) and significant time effect (Time effect: P = 0.0003) (Table 2).

## Discussion and Conclusion

The importance of lameness in cattle is considered as one of the most urgent health and welfare problems of dairy cows as well as one of the most significant economic issues for dairy industries [12,32,43]. Bovine lameness with the prevalence of about 15 - 40% in dairy cows [4,23] as well as frequency of about 80% due to distal hind limb lesions [37] is one of the most important welfare issues of high producing dairy cows [39,44]. To prevent and treat of such a crucial disorder, selection of an appropriate hind limb local anesthesia method would be essential [13,33,37]. Application of thermal stimuli to stimulate superficial (cutaneous) pain has been applied already in large animals such as horses [20,27,28,31] and small animals such as cat [2,34] or rabbit [5]. Also, there are some studies related to direct laser application in dairy cows [14,30]. Based on our results, the reason not to have a clear measurable response after application of mechanical pin pricks and force and especially heat nociceptive thresholds can be considerable. In our examination, to fix the right hind limb to the tilt table, approximately tight belt was used. Therefore, consequently, tourniquet induced ischemia and nerve dysfunction, the location of applied thermal and mechanical stimuli and so on could be the most probable effective reasons not to have a clear measurable response after administration of thermal and mechanical nociceptive stimulators. A general stress due to handling and restraint in lateral recumbency as well as induced hypoxia distally to the fixing belt of hind leg could be considerable. Some studies demonstrated that, application of tourniquet can cause systemic variations resulted in produced tourniquet-pain such as increasing the level of blood pressure as well as localized side effects such as ischemic changes beneath as well as distal to the tourniquet [18]. Following hypoxia resulted in ischemia, the affected muscles beneath and distal to the applied tourniquet will release some inflammatory factors which can induce pain [3,8,46]. Additionally to effect of induced tourniquet-ischemia on plasma lactate with increasing lactate concentration [11], the noticeable effect of produced ischemia could be its efficacy on nerves' function by decreasing nerve conduction velocities [6]. Subsequently, after 15 minutes of tourniquet ischemia, the sensory thresholds significantly increase which can be referred to the disappearance of large diameter fibers action potentials [30] Secondary to the tourniquet induced ischemia, nerves dysfunctions can be generated due to neuro-muscular injuries [26]. It has to be thought the rapid conducting myelinated Aδ fibers under normal circumstances could inhibit C fibers in relation to the transmission of pain, can be blocked by mechanical compression within ischemia period. Touch sansation cannot be perceived when painful sensation is produced at the same time. Therefore, showing no reaction following mechanical noxious stimuli before anesthesia application resulted in tourniquet ischemia as well as induced nerve damages which both could produce tourniquet pain would be expected in some dairy cows with a tight fixing belt in lateral recumbency on the surgical tilt table [16].

The location of nociceptive threshold application would play a crucial rule inducing clear measurable response after nociceptive thresholds. For instance, regarding application of wireless thermal threshold testing device to induce noxious thermal stimuli at coronary band of the bovine claw, it would be noticeable to mention that coronary band area could have a lowest reliability to generate constant clear reaction to the heat stimulation [27,28], it has been referenced to the coronary band skin's thickness in different animal species. Furthermore, blood flow could be effective to produce thermal threshold nociceptive responses as well [20]. In overall, thermal threshold nociceptive responses at coronary band could be influenced by skin thickness as well as less blood flow of this area. Therefore, the more distal parts of the hind leg as thermal stimulation area like coronary band can possibly be affected the heat nociceptive threshold responses by the temperature changes as well as blood flow [27,28].

Mechanical stimulation devices should be easy to use with the reproducible responses as a result of mechanical stimulations [8]. One of its limitations is that the stimulus as well as its application's rate must be same from trial to trial. the mechanical pressure nociceptive threshold results of second part of study for onset of anesthesia revealed that there is no difference between the speed of onset of anesthesia at lateral and medial flexor tendon as well as lateral and medial dorsal aspect of fetlock joint and also lateral and medial heel bulb following anesthesia with either nerve block or intravenous regional analgesia. However, regarding the mechanical pressure nociceptive threshold responses from the examined dairy cows in our study, full anesthesia could be obtained fifteen minutes following intravenous regional anesthesia in all six examined sites includes of lateral and medial heel bulb, lateral and medial dorsal aspect of fetlock joint as well as lateral and medial flexor tendon while nerve block anesthesia was started at those six different sites following anesthesia already. About our second examination, the onset of anesthesia can be faster about ten minutes at the skin of heel after our modified four-point nerve block anesthesia compared to intravenous regional anesthesia as well as seven and half minutes following anesthesia's application at the skin of dorso-lateral coronary band. Furthermore, the duration of anesthesia would be the same between two anesthesia techniques as there was not seen any significant difference fifteen and thirty five minutes following anesthesia with either four points regional nerve block or intravenous regional anesthesia. It seems, if we had a plan to increase numbers of nociceptive threshold stimulations after anesthesia with increasing the duration of lateral recumbency on surgical tilt table, that could be possible to get to know which anesthesia technique would be ended sooner. However, regarding the time which veterinarians would need to perform at the distal hind limb, realizing the duration of full desensitized distal hind limb more than thirty five minutes following anesthesia would be great. As the results of our first assessment revealed, full desensitized distal hind leg could not be significantly different between four-point nerve block anesthesia method and intravenous regional anesthesia technique at the lateral and medial heel bulb, lateral and medial flexor tendon as well as lateral and medial dorsal aspect of fetlock joint. In other words, according to mechanical pin pricks nociceptive threshold responses, intravenous regional anesthesia and four-point nerve block anesthesia could induce same adequate analgesia at lateral and medial heel bulb, lateral and medial flexor tendon as well as lateral and medial dorsal surface of fetlock joint. Therefore, there is no difference to choose intravenous regional or nerve block anesthesia to have a full desensitization at lateral and medial heel bulb, lateral and medial flexor tendon as well as lateral and medial dorsal surface of fetlock joint. However, based on our evaluated results related to onset of anesthesia, intravenous regional anesthesia could be started about ten minutes after anesthesia's application later than our modified four-point nerve block anesthesia at the dorso-lateral coronary band skin as well as soft skin of heel. Based on the results of our first study, application of intravenous regional analgesia or administration the modified four-point nerve block anesthesia does not have any remarkable difference to have a full or complete desensitized area at lateral and medial heel bulb, lateral and medial flexor tendon as well as lateral and medial dorsal aspect of fetlock joint. Nonetheless, the onset of anesthesia would be still superior following our modified four-point nerve block anesthesia compared to intravenous regional analgesia at both sites of dorso-lateral coronary band as well as soft skin of heel. Those recorded results demonstrated that before choosing the local anesthesia technique, first of all, that's better to identify the desired desensitized area before any intervention. By this strategy, the veterinarians would have a full anesthesia in the minimum period of time needed for distal hind limb's surgical interventions. In our study, Top cat metrology device to induce heat nociceptive stimuli was used. This device is designed to induce heat stimulation with the cut-off at 55°C in small animals. After some examinations on dairy cows, we decided to use. However, according to our results, 37.5% of examined dairy cows did not have any response even before anesthesia with either intravenous regional or modified four point nerve block anesthesia and 37,5% of dairy cows in one of their treated day had no response to thermal stimuli. General stress due to handling and restraint in lateral recumbency as well as induced hypoxia distally to the fixing belt of hind leg could be effective. Having no response following thermal nociceptive thresholds before anesthesia application in our study can be in agreement with Ashworth., *et al.* (2002) and Chabel., *et al.* (1990), stating that after hypoxia resulted in ischemia, affected muscles beneath and distal to the applied tourniquet can release some inflammatory factors to induce pain. Koga., *et al.* (2005) and Schaible and Richter (2004), explained that touch sansation cannot be perceived when painful sensation is produced at the same time. Thermal and mechanical noxious stimuli activate nociceptors of Aδ and C fibers in peripheral nerves and these fibers can be blocked by mechanical compression of tourniquet as well as tourniquet - induced ischemia. Therefore, showing no reaction following mechanical noxious stimuli before anesthesia application resulted in tourniquet ischemia as well as induced nerve damages would be expected in our examined dairy cows with the tight fixing belt. However, in spite of above studies, Moldaver (1954), highlighted that mechanical compression effect of tourniquet can eliminate the position sense, touch, vibration, motor and light pressure while warmth, coldth, pain and sympathetic sensations will not be affected under this mechanical compression and nerves distal to the tourniquet reply to electrical nociceptive thresholds. Although, application of wireless thermal stimulations have been developed nowadays [2] administration of such this stimuli should be considered in different species. In our experiment, Top cat metrology heat threshold testing device was used above the coronary band of the claw with a recognized thermal reaction of claw movement. That responses recorded, only 62.5% of dairy cows did not have a measurable response to such this heat stimulator under our experimental conditions such as application of thermal nociceptive threshold stimuli on the lateral recumbent dairy cows with an approximately tight fixing belt to fix legs to the surgical tilt table. Another effective considerable factor is that, our wireless thermal threshold testing device was applied to induce heat stimuli at coronary band of the bovine claw (effect of the location). Our results can be in agreement with Poller., *et al.* (2013), highlighting, thermal nociceptive measurements at the coronary band area could have the lowest reliability to generate constant clear response. That effect can be referenced to the coronary band thickness in different species of animals. Furthermore, blood flow could also be effective to produce thermal threshold nociceptive responses [20]. In overall, thermal nociceptive threshold responses at coronary band could be influenced by skin thickness as well as less blood flow of this area. Therefore, the more distal parts of the hind leg as thermal stimulation area like coronary band could be possibly affected the heat nociceptive threshold responses by temperature as well as blood flow [27,28]. The tissue damage resulted in thermal nociceptive threshold testing should be taken into an account as another effective factor. A valid pain measurement means should not cause tissue damage while thermal nociceptive threshold testing devices are more likely to cause small skin lesions than mechanical threshold testing systems [19] our study, the results of application of thermal nociceptive thresholds at coronary band of cattle claw in our study are in agreement with Natalini and Robinson (2000) as well as Natalini., *et al.* (2006) mentioning due to inadvisable increasing cut-off thermal thresholds, superficial skin lesions can occur. Moreover, daily variations of environmental temperature can affect thermal nociceptive threshold testing not only within some animal related factors such as skin temperature or presence of skin moisture but also can interfere with heat stimulation device directly [24,34,35]. In conclusion, heat nociceptive threshold devices are the third most reliable stimulators after electrical and mechanical stimulators to measure pain in dairy cows base on their application's criteria.

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