



## Comparison of alternative disbudding methods with hot-iron dehorning of goat kids



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### ABSTRACT

Goat kids are frequently disbudded in intensively farmed units to reduce the number of accidental injuries to other animals and farm workers due to horn growth. The most commonly used method is thermal disbudding which is increasingly causing animal welfare concerns. Therefore the objective of the present study was to evaluate alternative disbudding methods either by injecting clove oil or its synthetic analogue isoeugenol.

In this study 40 male Saanen goat kids aged between one and five days were treated using 4 different methods ( $n = 10$  each): injection of 0.2 mL clove oil, 0.2 mL isoeugenol, 0.2 mL isotonic NaCl solution and thermal disbudding. For thermal disbudding general anesthesia was performed. Horn growth of all goat kids was measured. Computer tomography (CT) of the horn bud region and histological examination of biopsy samples taken from the horn bud region were performed. Additionally, saliva cortisol concentrations were measured.

Highest success rate was reached with thermal disbudding and disbudding with isoeugenol followed clove oil. Scurs growth after disbudding appeared in 11 horn buds (4 thermal disbudding, 4 clove oil, 3 isoeugenol). CT images of animals after thermal disbudding showed complete destruction of the horn buds but also damage to the frontal bones. CT images of animals after clove oil or isoeugenol injection demonstrated partial destruction or malformation of the horn buds. Purulent osteomyelitis was detected after thermal disbudding ( $n = 2$ ) and clove oil treatment ( $n = 1$ ). Histological examination of biopsy sample showed different changes of skin, dermis and epidermis. A significant difference of between the cortisol concentrations before treatment and 10 minutes after treatment was only found in the isoeugenol group ( $P = 0.015$ ). In the other groups, the increase was not significant. Likewise, only the isoeugenol group showed significantly higher cortisol concentrations ( $p = 0.019$ ) following treatment in comparison with the control group (NaCl).

Highest efficacy was reached with thermal disbudding and with isoeugenol followed by clove oil. Disbudding goat kids with isoeugenol caused less tissue damage than thermal disbudding. Injection of clove oil or isoeugenol may therefore be considered as an alternative to thermal disbudding. Future research is required to evaluate the behavioral responses of goat kids to these methods of disbudding. In addition,

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more research is needed to optimize the injection technique and to validate the growth of scurs after disbudding in goat kids.

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## Introduction

Disbudding is a commonly performed procedure in dairy goat kids. In young goats kids the horn buds are still movable and not yet attached to the frontal bone (Dyce et al., 2009). Disbudding is most commonly performed using a hot iron (Ingvast-Larsson et al., 2011). Since heat destroys the horn bud tissue, a multimodal approach for pain management involving administration of local anesthetics in combination with non-steroidal anti-inflammatory drugs is required. In a number of countries general anesthesia is required. However, even if pain relief is provided during disbudding, the tissue damage caused by thermal disbudding leads to long-term pain and in some cases also to local infections (Adcock and Tucker, 2018; Sutherland et al., 2019a). Procedures causing unjustifiable pain or distress in animals are generally not allowed in Europe. However, disbudding is allowed in a number of countries under special national legislation. While disbudding kids is generally forbidden in Germany, in Austria, Switzerland and the UK veterinarians are allowed to perform disbudding following specific regulations (Bundesgesetzblatt für die Republik Österreich Nr. 118, 2004, Tierschutzgesetz TschG § 6., Tierschutzgesetz (TschG) Art. 16., Veterinary Surgeons Act 1966). Wagmann et al. (2018) described that ketamine-xylazine mixture for anesthesia during disbudding, which was administered by trained and certified farmers was not sufficiently effective. The results of Alvarez and Gutierrez (2010) indicate that goat kids suffer from acute stress during thermal disbudding. They described that the procedure induces an acute increase of cortisol concentration after disbudding, which lasted for 2–3 hours. This also suggests that anesthesia and analgesia is required to avoid pain and stress. Goat kids that were anaesthetized using a nerve block with lidocaine showed less behavioral reactions (Wagmann et al., 2018). Nfor et al. (2016) described that plasma cortisol levels in thermal disbudded goat kids were significantly higher than those that received lidocaine and meloxicam. Ingvast-Larsson et al. (2011) received identical results as Nfor et al. (2016). Lidocaine was injected as ring block which appeared to cause more pain than handling alone and may not reduce pain associated with thermal disbudding sufficiently. Hempstead et al., (2020a) also considered a lidocaine ring block not feasible for thermal disbudding in goat kids. Additionally, they found no evidence that meloxicam reduced acute pain within 30 min of disbudding. Hempstead et al. (2020b) described that cortisol concentrations were lower in thermally disbudded goat kids which were anaesthetized using isoflurane with or without meloxicam compared to those disbudded without pain medication. However, it has to be mentioned that all types of anesthesia or/and anti-inflammatory drugs influence the cortisol secretion.

Wounds after thermal disbudding in goat kids take about 7 weeks (50 ± 8 days) to re-epithelialize and damaged tissue during healing is more sensitive than re-epithelialized tissue meaning that wounds remain painful over this long period (Alvarez et al., 2019). In addition, skull bone injury (Hempstead et al., 2018d), cerebral infarction and meningoencephalitis following hot-iron disbudding have been observed in goat kids (Thompson et al., 2005).

Clove oil has been used for many years in dentistry as topical anesthetic drug (Markowitz et al., 1992) and has also cytotoxic, antibacterial and anticarcinogenic properties (Chaieb et al., 2007). Clove oil is also used as anesthetic drug in fish (Sladky et al.,

2001). The combination of both cytotoxic and analgesic properties make clove oil a drug that could be feasible to destroy the horn buds as alternative to the thermal disbudding. Clove oil injection in the horn bud region as a novel method of disbudding goats has been first described by Molaei et al. (2015). This study showed that the injected clove oil completely stopped horn growth in goat kids. However, the study was performed in a very small number of animals. In another study disbudding wounds were closed and covered with dry blackened skin 6 weeks after clove oil injection (Hempstead et al., 2018a). Hempstead et al. (2018b) also evaluated cortisol concentrations after disbudding goat kids. From these 2 studies it was concluded that clove oil may serve as an alternative to thermal disbudding because it causes less tissue damage and was followed by faster wound healing (Hempstead et al., 2018e). However, a recent paper concluded that further research is needed to refine the technique particularly its administration before it can be widely used in practice (Sutherland et al., 2019a).

The present study was performed to evaluate if alternative disbudding methods may cause less tissue injury and pain than thermal disbudding. Injection of clove oil or isoeugenol (the synthetic derivative of clove oil) or isotonic saline solution were performed and compared to thermal disbudding in goat kids. The study included measurements of horn growth, computer tomography (CT) of the horn bud region, histological examination of biopsy samples taken from the horn bud region and saliva cortisol concentration pre and post treatment.

## Hypotheses

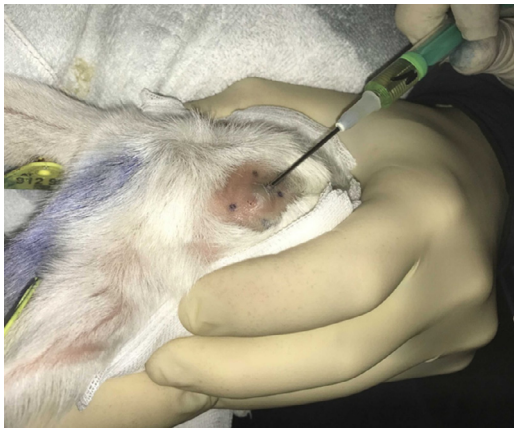
Clove oil and isoeugenol injections are equally effective in the suppression of horn growth as thermal disbudding but cause less pain and distress and are followed by faster wound healing.

The alternative treatments result in less severe tissue damage than thermal disbudding which may even cause frontal bone ore cerebral damage.

## Animals, materials and methods

The study was conducted on 40 male Saanen goat kids aged 1 to 5 days (mean 2.45 d, SD 1.11 d) in spring 2018 and was approved by the institutional ethics and animal welfare committee of the Veterinary University of Vienna in accordance with good scientific practice guidelines and national legislation (GZ 68.205/0049-WF/V/3b/2016). Given the fact that horn growth in male kids is stronger than in female kids, only male kids were used. If clove oil and/or isoeugenol injection prevents horn growth in male kids it is reasonable to believe that the method would be successful in female goat kids too.

- All animals were housed with their dams in a dairy goat farm in Upper Austria. Kids were weighed and identified by individual ear tags. The animals were assigned at random by drawing lots to one of the four groups (10 kids per group). Both horn buds of each animal were treated with the same method.
- Group 1 - clove oil: kids were injected with 0.2 mL (volume used by Molaei et al., 2015) of clove oil (*Syzygium aromaticum*; synonym: *Eugenia caryophyllata*; Herba Chemosan Apotheker-AG, Austria) laterally to the center of each horn bud at a 45° angle



**Figure 1.** Goat kids were injected with 0.2 mL clove oil, isoeugenol or NaCl laterally into the center of each horn bud at a 45° angle.

between nasal bridge and ear using a 16 G needle (BOVIVET 16 G x 1-1/2 " 1.6×38 mm, Jørgen KRUISE A/S, Denmark) (Fig.1).

- Group 2 - isoeugenol: kids were injected with 0.2 mL of isoeugenol ((C<sub>10</sub>H<sub>12</sub>O<sub>2</sub>; 2-methoxy-4-prop-1-enylphenol) Merck KGaA, Germany) applying the identical procedure like group 1.
- Group 3: thermal disbudding: kids were thermally disbudded using a hot iron (Buddex, Albert Kerbl GmbH, Germany) during general anesthesia (xylazine 0.05 mg/kg bodyweight i.m.; ketamine 10 mg/kg bodyweight i.m.). The horn buds were removed after the hot iron had been applied.
- Group 4 - control group: kids were injected with 0.2 mL of NaCl (Kochsalz "Braun 0.9% - Infusionslösung", B. Braun Austria GmbH, Austria) applying the identical procedure like group 1.

#### Measurement of horn growth

Goat kids were observed concerning horn growth over a time period of 6 months. Horn growth (length in cm) was measured 4 times (1, 2, 4 and 6 months after treatment) using a gauging tool.

#### Computer tomography (CT) investigation of the horn bud region

CT images of 11 animals were taken (3 animals from the control group, 3 after thermal disbudding, 5 after chemical disbudding (3 clove oil and 2 isoeugenol). Computed tomography of the head was performed 21 d after treatment using a 16-slice scanner (SOMATOM Emotion 16, Siemens Healthcare, Erlangen, Germany). Animals were anaesthetized (xylazine 0.05 mg/kg bodyweight i.m., ketamine 10 mg/kg bodyweight i.m.) and placed in sternal recumbency. Technical settings were 110–130 kV and 144–200 mAs. Effective slice thickness was 0.6 – 0.75 mm. Collimation was 16×0.6 mm. The scans were reconstructed using both soft tissue and bone algorithms. Early (arterial), coupled with early (60 sec post injection) and late venous (100 sec post injection) contrast series (600 mg iodine per kg body weight intravenously, 1.5 ml/s) were performed in all cases. CT images were stored in a picture archiving and communication system. Multiplanar reconstructions and surface models were calculated using a syngo MultiModality Workplace (JiveX; Visus Health IT GmbH, Bochum, Germany).

CT images were reviewed without knowledge of the treatment method by one observer (S.K.). Observations regarding presence or absence of the horn bud, or intervention-correlated soft or bone tissue changes were noted.

#### Histological examination of biopsy samples from the horn bud region

Punch biopsy samples (10 mm diameter) of the horn bud region were sampled from 20 goat kids (one horn bud per animal, 5 per each group) on the same day as the CT-investigations. Biopsy sampling took place under general anesthesia and application of NSAID (1.4 mg Carprofen per kg bodyweight; s.c.). Biopsy sample were taken from the center of the horn bud, or in case of horn growth, as close as possible to the center of the horn bud.

The samples were fixed in 10% neutral buffered formalin and if necessary decalcified (Decal, StatLab, McKinney, Texas, USA) before embedded in paraffin-wax, sectioned at 2 µm and routinely stained with hematoxylin and eosin (HE) for the histopathological examination. The biopsy samples were examined in the order they were taken, the examiner (DT) was also not aware of the treatment.

#### Salivary cortisol

After clinical examination saliva from each goat kid was collected pre and post treatment. Saliva was collected with a Salivette (SalivaBio Children's Swab (SCS), Salimetrics LLC; USA) fixed at an artery clamp. Sampling was performed immediately before treatment as well as 10 and 60 minutes after treatment. All salivettes were placed in the proposed double cylinder of Salivette Cortisol (Sarstedt AG and Co. KG, Germany) directly after saliva sampling and stored frozen (-80°C) until cortisol measurement.

Saliva samples collected in double cylinders were thawed in a water bath at 38°C for 20 minutes and afterwards centrifuged at 2500 g for 10 minutes. After centrifugation 50 µl of each sample was transferred to Biorad tubes and 450 µl enzyme immunoassay (EIA) buffer added (diluted 1:10). Determination of cortisol was carried out with a cortisol EIA (Palme and Möstl, 1997), which has already been used in calves' saliva (Wagner et al., 2013). The laboratory was blinded to the used disbudding method.

#### Statistical analysis

The horn buds are considered the experimental units resulting in a sample size of 20 horn buds per group. Descriptive and comparative statistics were calculated using Microsoft Excel 2010 and IBM SPSS Statistics (Version 24.0). Correlations between different methods were performed by U-test, Wilcoxon, Kruskal-Wallis-Test and Mann-Whitney test. For all analyses, a p-value < 0.05 (5%) was considered as significant. In addition a post hoc power analysis (alpha 0.05) for the comparison of groups was performed.

#### Results

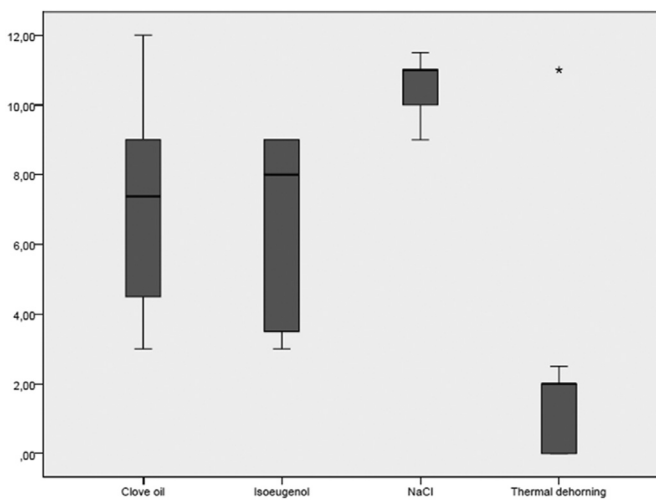
Of the 40 kids enrolled in this study one died 3 month after treatment (group thermal disbudding) due to a colic (no further postmortem diagnosis available). At this time the horn bud was 0.5 cm on both sides.

#### Horn growth

Horn growth was measured 4 times and is shown in detail in (Table 1) and (Figure 2). 6 month after disbudding horn growth of group 1 (clove oil) was between 2 cm and 12 cm (mean 7.22 cm; SD 3.21 cm), of group 2 (Isoeugenol) 3 cm and 9 cm (mean 6.44 cm; SD 2.86 cm) of group 3 (thermal disbudding) between 0 cm and 3 cm (mean 2.25 cm; SD 3.44 cm) and of group 4 (NaCl) between 9 cm and 12 cm (mean 10.50 cm; SD 0.83 cm). Significant differences between isoeugenol treatment and control group ( $P=0.000$ ), between clove oil treatment and thermal disbudding ( $P=0.03$ ) and between thermal disbudding and control

**Table 1**  
Measured horn growth (length in cm) of all treated animals 1 month, 2 month, 3 month and 6 month post treatment. + died 3 month post treatment.

ID	treatment	horn growth in cm 1 month post treatment		horn growth in cm 2 months post treatment		horn growth in cm 3 months post treatment		horn growth in cm 6 months post treatment	
		left	right	left	right	left	right	left	right
1	clove oil	1	0	3	1	9	9	9	9
2	clove oil	0	0	5	1	3	3	5	4
3	clove oil	1	2	3	5	5.5	6	05.Mai	6
4	clove oil	2	2	3	2	11	11	11	11
5	clove oil	2	2	3	2	5	5	5	5
6	clove oil	1	0	2	0	6	1	6	2
7	clove oil	1	2	3	4	9	9	9	9
8	clove oil	0.5	0.5	2	2	2	2	3	3
9	clove oil	3	3	4	4	12	12	12	12
10	clove oil	2	2	3	3	9	9	9	9
11	isoeugenol	0.5	0.5	3	3	9	9	9	9
12	isoeugenol	2	2	3	3	9	9	9	9
13	isoeugenol	1	1	3	3	9	9	9	9
14	isoeugenol	0.5	0	0.5	0	3	2	5	3
15	isoeugenol	2	2	3	2	9	9	9	9
16	isoeugenol	1	0.5	1.5	1	2	3	3	4
17	isoeugenol	1	1	3	3	8	8	8	8
18	isoeugenol	1	1	1	1	2	2	3	3
19	isoeugenol	0	0	0	0	2	2	3	4
20	isoeugenol	0	0	0	0	0	0	0	0
21	thermal	0	0	0	0	0	0	0	0
22	thermal	0	0	0	1	2	3	2	3
23	thermal	0	0	0	0	2	2	2	2
24	thermal	0	0	0.5	0.5	2	2	2	2
25	thermal	0	0	0	0	0	0	0	0
26	thermal	0	0	0	0	0	0	0	0
27+	thermal	0	0	0.5	0.5				
28	thermal	0	0	0.5	0.5	0.5	1	0.5	1
29	thermal	4	4	4	4	11	11	11	11
30	thermal	1	1	2	2	2	2	2	2
31	control	4	4	6	6	11	11	11	11
32	control	4	4	6	6	11	12	11	12
33	control	4	4	6	6	11	11	11	11
34	control	4	4	6	6	11	11	11	11
35	control	4	4	7	7	10	9	10	9
36	control	4	4	7	7	11	11	11	11
37	control	4	4	7	7	11	11	11	11
38	control	4	4	6	6	10	10	10	10
39	control	4	4	6	6	10	10	10	10
40	control	4	4	4	4	9	9	9	9



**Figure 2.** Horn growth 6 month after disbudding in centimeters compared between the different groups.

group ( $P=0.01$ ) were found. Although there were no significant differences in horn growth between isoeugenol treatment and

thermal disbudding, ( $P=0.05$ ) a tendency towards a higher efficacy of thermal disbudding was obvious. A *post hoc* power analysis (alpha 0.05) for the comparison of these two groups calculated a power of 94.1%. Between clove oil treatment and isoeugenol treatment ( $P=0.42$ ) and clove oil treatment and control group ( $P=0.14$ ) no significant difference could be verified.

Goat kids developed 11 scurs (4 thermal disbudding, 4 clove oil, 3 isoeugenol). Scurs after thermal disbudding were 3 cm, 1 cm, 0.5 cm, 0.5 cm long, scurs after clove oil injection 5 cm, 4 cm, 5.5 cm, 11 cm and scurs after isoeugenol injection 3 cm, 3 cm and 9 cm long.

As examples the situation 2 month after isoeugenol treatment (Figure 3) and 2 month after clove oil treatment is shown (Figure 4). The status 6 month after treatment with isoeugenol (1) and clove oil (2 and 3) is shown in (Figure 5). This figure shows different horn growth and horn length in 2 animal after isoeugenol (1) and clove oil (2 and 3) treatment.

*CT-examination of horn bud region*

CT images of the three control animals were evaluated as normal and all control kids showed similar developmental stages of their horns. CT images after thermal disbudding showed complete destruction of the horn buds. Additionally the bone destruction in-





**Figure 3.** Status 2 month after isoeugenol treatment. There horn bud region show dry skin lesions and no horn growth can be observed.



**Figure 4.** Status 2 months after clove oil treatment. The horn growth differ between left and right.

involved parts of the frontal bones in all 6 examined horn buds. CT images of 5 animals after clove oil and isoeugenol treatment showed only partial destruction or malformation of horn buds in 4 animals. In one animal the horn buds were completely destroyed. None of those 5 animals showed an involvement of the frontal bones (Figure 6).

#### Histological examination of biopsy samples from the horn bud region

In total 20 biopsy samples were histopathologically examined; in 3 cases a decalcification was necessary. 8 samples showed normal unchanged skin, samples belonging to three different groups (1 clove oil, 2 isoeugenol, 5 control group). The remaining 12 samples showed a varying degree of necrosis and purulent inflammation (Figure 7). The affecting epidermis and dermis was affected in nine cases (4 clove oil, 3 thermal disbudding, 2 isoeugenol). 1 sample (thermal disbudding) showed a necrotic epidermis but vi-

tal dermis, one sample had a necrotic epidermis (isoeugenol) and 1 (thermal disbudding) a necrotic dermis (thermal disbudding). A purulent osteomyelitis as well as proliferating fibroblasts and granulation tissue at the margin of the dermis were detected in three samples (2 thermal disbudding, 1 clove oil).

#### Salivary cortisol

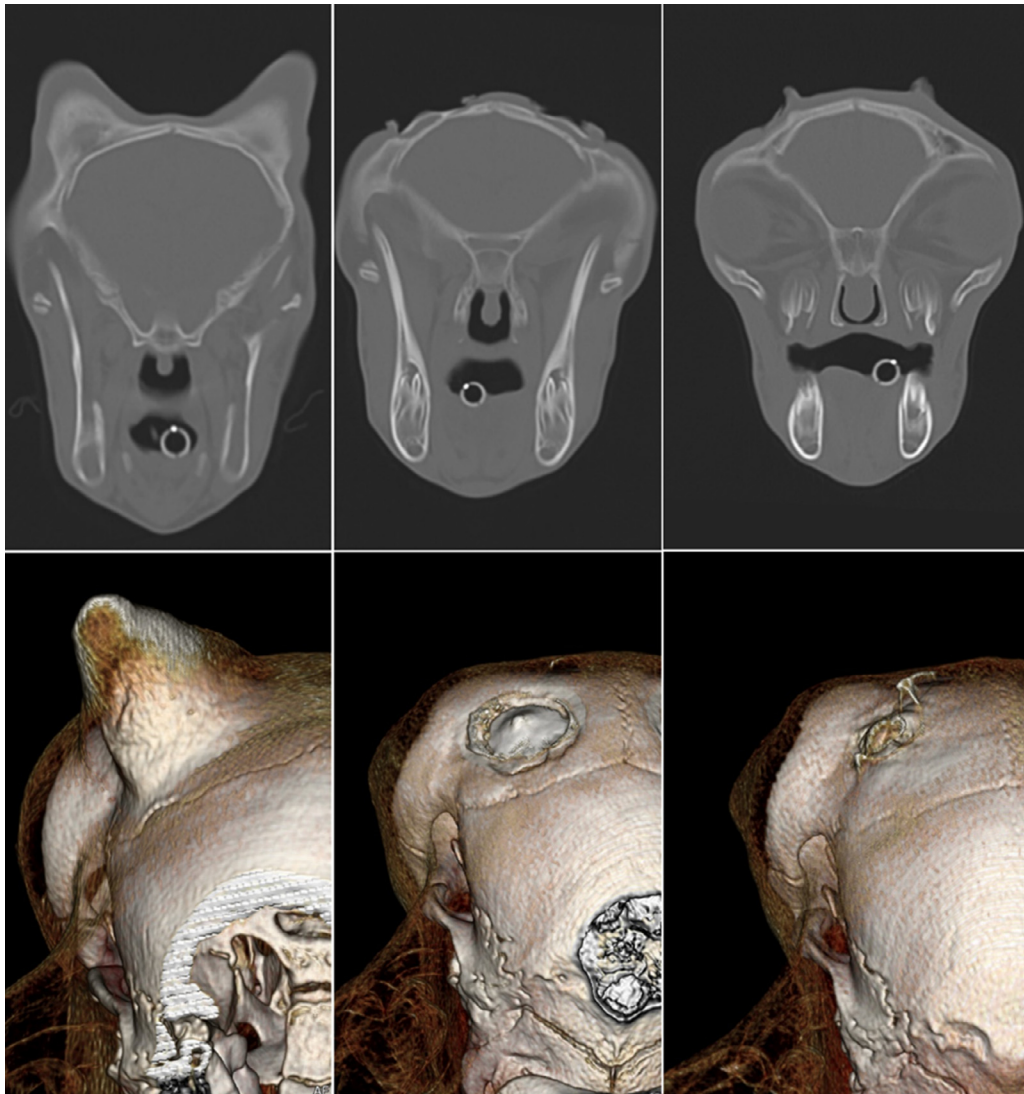
A significant increase in cortisol concentrations 10 minutes after intervention was only present in the isoeugenol group ( $P=0.015$ ). However there was the tendency that cortisol concentration were numerically higher 10 minutes after treatment and lower 60 minutes in all groups (Figure 8). There was 1 outlier (16.51 ng/ml) in the NaCl group before treatment. Another 3 outliers were found in the clove oil group with cortisol concentrations of 11.30 ng/ml before treatment and 19.36 ng/ml and 6.38 ng/ml 60 minutes after intervention.

#### Discussion

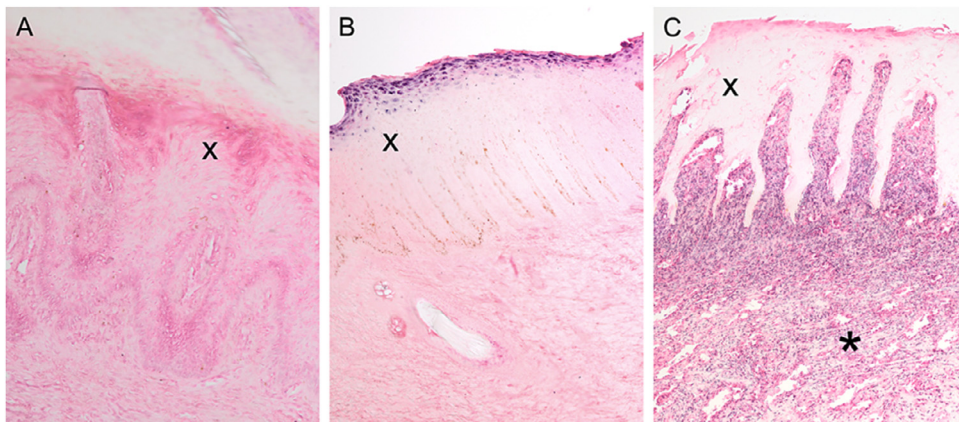
Currently there is limited research on the use of clove oil and isoeugenol for disbudding calves and goat kids. Molaei et al. (2014) and Schoiswohl et al. (2020) described injection of clove oil or isoeugenol for preventing horn growth in calves. Molaei et al. (2015) reported successful application of clove oil in goat kids. However the number of involved animals was low in the study. Meanwhile a number of studies describe the need for refinement of the technique (i.e., administration methods to improve efficacy) before clove oil injection could be considered an alternative to thermal disbudding (Hempstead et al., 2018a; Hempstead et al., 2018b; Hempstead et al., 2018c; Sutherland et al., 2019a; Sutherland et al., 2019b, Still Brooks et al., 2021).



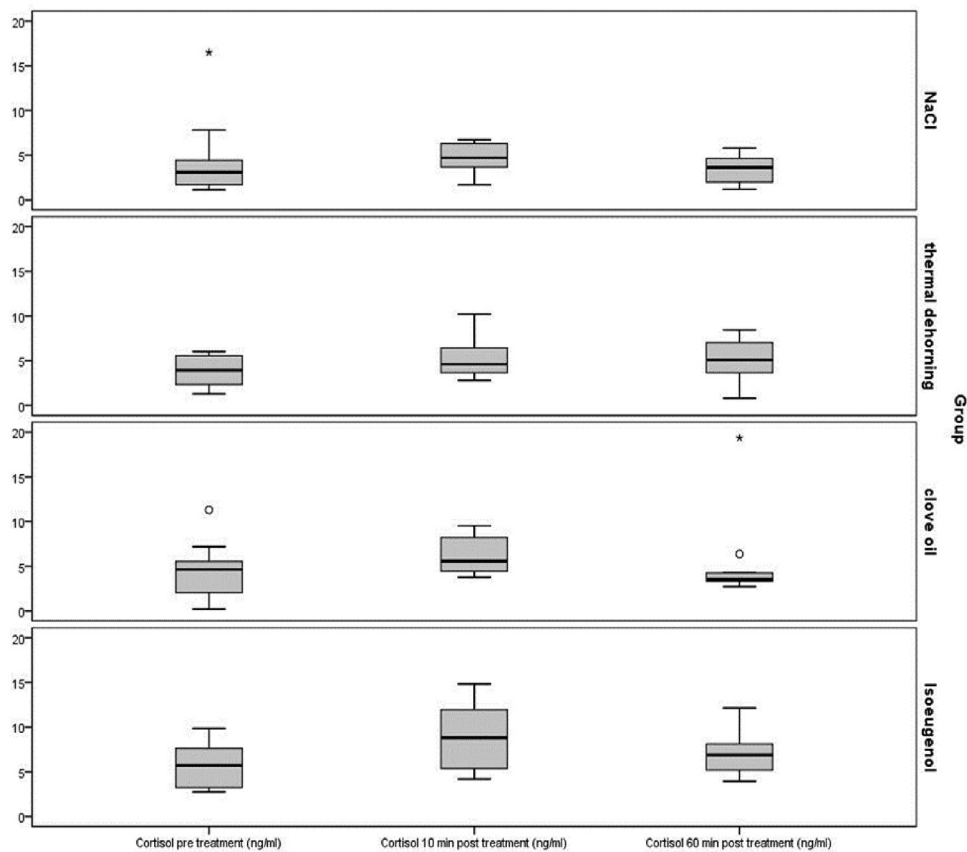
**Figure 5.** Status 6 month after treatment; 1: isoeugenol, 2 and 3 clove oil. Different horn growth and horn length at the same animal after isoeugenol (1) and clove oil (2 and 3) treatment.



**Figure 6.** left: CT images of control animal showing normal developmental stage of their horn buds, middle: CT images after thermal disbudding +showed complete destruction of the horn bud and the inclusion of the frontal bone, right: CT images after clove oil and isoeugenol treatment showed partial destruction of the horn buds.



**Figure 7.** A: Norm skin biopsy sample with hyperplastic epidermis and cornified epithelium (asterisk); B: necrotic epidermis and dermis; C: necrotic epidermis and dermis with a severe infiltration with degenerated neutrophils; (hematoxylin and eosin).



**Figure 8.** Saliva cortisol concentrations (ng/ml) pre and post treatment. The interquartile range includes around half of the measured values at the observed measurement time. A significant difference of the cortisol concentration before and 10 minutes after intervention was shown in the isoeugenol group ( $P=0.015$ ).

Schoiswohl et al. (2020) reported that the efficacy of clove oil and isoeugenol for disbudding calves is mainly dependent on the dosage and that the calves need to be treated at a very young age (less than 4 days old) to facilitate high efficacy. In contrast to Molaei et al. (2014 and 2015) and Schoiswohl et al. (2020) in calves, Hempstead et al. (2018b) reported in goat kids that clove oil treatment appeared to be less effective at preventing horn growth than cautery disbudding because of a higher incidence of scurs and unaffected horn growth. Clove oil injection was associated with several unexpected and severe complications including necrosis, temporary paresis, skull defects, meningitis and death in 1 of the latest published studies (Still Brooks et al., 2021). Still Brooks et al. (2021) stated that none of the alternative methods of disbudding provided a feasible option over heat cautery to improve animal welfare. In the study presented here scurs were also observed after thermal disbudding, clove oil and isoeugenol treatment. Although there was no significant difference in horn growth between isoeugenol treatment and thermal disbudding more extensive scur growth after isoeugenol and clove oil is a major problem which likely prevents the use of the method.

Clove oil and isoeugenol were injected laterally into the horn bud region using a needle, whereas thermal disbudding involved pressing a cautery iron directly on the horn bud. Consistent administration of the complete volume of clove oil or isoeugenol into the center of the horn bud which is the correct location may not always have been achieved. Due to the difficulties restraining the kids during application of clove oil or isoeugenol in some cases the needle may have been in a slightly incorrect location. It seems possible that the development of an applicator facilitating the injection of a consistent volume at the correct location may

improve the efficacy (Hempstead et al., 2018). Incorrect administration or an insufficient volume may lead to scurs or unaffected horn growth. To our best knowledge there are no other studies published which described CT examinations after injection of clove oil and isoeugenol. The CT images of the control animals showed normal development of the horn buds. CT images of animals after thermal disbudding showed complete destruction of the horns bud but also extensive damage to the frontal bones. The frontal bone of goat kids, unlike that of calves, is thin and the frontal sinuses are not developed at this age. Especially if hot iron disbudding is performed to vigorously it may not only cause thermal damage to underlying bone but also to meninges and even the brain (Wright et al., 1983; Thompson et al., 2005). In the present study thermal disbudding was performed very carefully according to the manufactures instructions; yet partial damage of frontal bones was still present. CT images of animals after clove oil and isoeugenol treatment showed no or only very mild changes of the frontal bones. After thermal disbudding scur growth usually result from inadequate application of the hot iron in an effort to reduce the risk to injure the frontal bone and brain (Dawson et al., 2007).

The biopsy samples consisted of hairless skin with hyperplastic epidermis indicating that the punch biopsy was correctly positioned. The cytotoxic effect of clove oil and eugenol has been demonstrated by Prashar et al. (2006) or Molaei et al. (2015). *In vitro* using cell cultures of 3 different cell types (153BR fibroblasts, human normal dermal fibroblasts and human dermal endothelial cell line). These authors demonstrated a decrease of cell viability with increasing clove oil concentrations (Prashar et al. 2006). It is assumed that the cytotoxicity is directed at the cell membrane leading to necrosis or apoptosis, two mechanisms that can



occur simultaneously and may not be distinguishable microscopically. In the study of Molaei et al. (2015) necrosis of horn bud was detectable in animals that were treated with clove oil. Whereas in their study only a mild infiltration with neutrophils was present, some of our samples showed a severe neutrophilic reaction. This could be due to the fact that in our study, samples were taken 21 days after the treatment vs., 5 and 10 days respectively in the study of Molaei et al. (2015). As expected, osteomyelitis was mainly diagnosed in samples taken after thermal disbudding but also in 1 sample from the clove oil group.

Comparisons of cortisol concentrations between different groups have to be discussed carefully because in thermally dehorned kids general anesthesia had to be applied and the anesthetic drugs influence cortisol secretion (Hefti, 2010). We believe it is worth mentioning that the authors gained the subjective impression that the application of xylazine was more important for decreasing pain reaction than the nerve block. Administration of non-steroidal-anti-inflammatory drugs also reduced signs of pain significantly, but the higher cost of analgesic drugs means that they are less frequently used in field practice (Wagmann et al., 2018). There was an increase in cortisol concentrations 10 minutes after intervention in all 4 groups, thus restraint by itself could be an additional reason for the increase. Furthermore, injection of NaCl in the control group may have led to some level of stress or discomfort similar to injection of clove oil or isoeugenol, as suggested by behavioral observations during the treatment (Frahm et al. 2020). In the control group (NaCl) the mean value 60 minutes after treatment was lower than the mean value before treatment, suggesting that the value before injection was somehow influenced by pre-sampling handling of the kids and it had returned to basal values after 60 minutes. Although like Hempstead et al. (2018a) we were not able to differentiate whether the elevated cortisol concentrations were in response to the action of the drug to the cells (necrosis) or the displacement of tissues by the application of the clove oil or isoeugenol. In contrast to the present study, Hempstead et al. (2018c) described that clove oil caused similar amount of pain and a similar behavioral response to cautery disbudding.

## Conclusions

Based on our results disbudding goat kids with isoeugenol causes less tissue damage than thermal disbudding. Best dehorning success was reached with thermal disbudding and using isoeugenol. Clove oil or isoeugenol can be considered an alternative method to thermal disbudding, because they result in less tissue damage. A major problem is the growth of scurs after clove oil and isoeugenol injection in some animals. More research is needed to optimize the administration technique to improve success rate after clove oil and isoeugenol treatment. We suggest that in any further evaluation of these methods behavioral and other indicators of goat kid welfare should also be monitored.

## Ethical statement

The study was conducted on 40 male Saanen goat kids aged 1 to 5 days (mean 2.45 d, SD 1.11 d) in spring 2018 and was approved by the institutional ethics and animal welfare committee of the Veterinary University of Vienna in accordance with good scientific practice guidelines and national legislation (GZ 68.205/0049-WF/V/3b/2016).

## Cited References

Council of Europe., 2018, Cozzi et al., 2015

## Conflict of interest

This manuscript has not been published and is not under consideration for publication elsewhere. We have no conflicts of interest to disclose.

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