Physiological and Blood Response in Dairy Goat Kids Disbudding by Subcutaneous Injection of Eugenol and Cautery

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Abstract

Background

Eugenol (EG) is an effective factor in disbudding by clove essential oil. Therefore, this study was conducted to investigate the hematological and clinical effects of disbudding using EG in goat kids. The aim of this study was to compered the stress level, blood biochemical parameters, and some vital organ enzymes in goat kids following disbudding by eugenol and cautery. The 25 Raieni (Cashmere) goat kids (5-day-old) were randomly assigned to five equal groups (n=5).

Results

The cortisol serum level immediately after disbudding in group 2 was 7.94±0.7 μg/dL, vs. group 4, 6.34±0.55 (mean ± SE), immediately after disbudding respiratory rate in group 3 was 77±16.97 vs. group 1, 108±28.28, also pulse rate in group 3 was 239.4±28.26 vs. group 1, 284±38.36; and rectal temperature showed no significant difference ($P<0.05$).

Conclusion

According to the results disbudding by EG induced less stress and pain compared to disbudding by cautery in goat kids ($P<0.05$). Analysis of specific blood serum enzymes represented no side effect on vital organs in EG injection. Our data suggest that EG should be a suitable alternative to old and outdated or painful routine methods. Also, it is more economical than other methods.

Name of the registry

Iran National Committee for Ethics in Biomedical Research

Trial Registration Number

IR.UK.REC.1398.001

Date of registration

01/02/2018

URL of trial registry record

https://ethics.research.ac.ir/ProposalViewEn.php?id=58428

Background

Disbudding is standard procedure to reduce animal itself, other farm animals, and farmhand injuries, as well as reducing the space required.[1,2] Thermal disbudding like cautery is a routine and at the same times the painful practice used for dairy goat kids disbudding.[3] However, although farmers are responsible for minimizing the pain of animals, but for economic reasons or lack of knowledge of pain effective methods of pain moderation, this process is usually performed without effective pain relief.[4-6] Except for the painful method, misuse and placement of the cautery over time will cause severe burns in the area and due to the young age of the goat kids at the time of use of this method and not complete formation of the frontal bone, the possibility of meningitis following this procedure is very high[7] Topical agents used in other farm animals such as potassium hydroxide, sodium hydroxide, calcium hydroxide, and calcium chloride either lack the desired effect in goat kids or create more severe disadvantages like damages to structures adjacent to the frontal horns, eye, ear, frontal bone and even brain. The other complication of disbudding by these techniques is the horn buds' regrowth.[8] It also prevents the production of hornless goats from genetic manipulation because of the association of intersex recessive genes with those of the hornless.[9] Consequently, novel alternative should be investigated to improve goat kids' welfare.

Stress and pain stimulate the sympathetic and parasympathetic nerves, and subsequently result in changes in clinical parameters such as body temperature, pulse rate, and respiratory rate (TPR)[10] which is usually absent in the similar studies and not much attention is paid to these changes together with cortisol. In addition to physiological and behavioral changes measurement[11,12] in animals to measure pain, serum cortisol levels can also be used. Cortisol is the main glucocorticoid hormone that is released in response to pain or stress. Cortisol level in the serum can be measured for the hypothalamic-pituitary-adrenal (HPA) axis stimulation, which is activated in the painful conditions.[10,13] Due to the response of serum cortisol levels to other endogenous and exogenous stimuli such as sexual cycle, restrain, blood sampling, as well as circadian, interpretation of the results of its measurement requires high accuracy and should be along with other measured parameters.[1,14]

One of the novel methods that has been introduced is clove oil subcutaneously (SC) injection.[15] Studies on the chemical structure of clove essential oil have shown that 72-95% is made up of eugenol (EG), so it is a probable effective factor in disbudding by the clove essential oil.[16,17] EG as a chemical component (4-allyl-2-methoxyphenol–C10H12O2) is found in essential oil taken from some herbal species like Syzygium aromaticum (clove), Cinnamomum verum, and Pimenta racemose.[18,19] Anti-inflammatory, anesthetic, and anti-oxidant effects of EG have been well documented in the previous studies.[20-22] Inhibition of cyclooxygenase type II enzyme causes anti-inflammatory activity of EG, and selective binding to capsaicin receptor makes anti-nociceptive effects of EG.[20,23] Also, some studies have shown that EG have anti-bacterial and anti-inflammatory effects.[24,25] EG has been used as a core in folk medicine in ancient civilizations. It has also been used in oral hygiene, food flavor, perfume production, anesthetic productions in fish, and dental analgesia.[25]

Our hypothesis was that goat kids disbudding by EG as a novel procedure might less painful than other traditional routine method in lack of any analgesic or anesthetic agent. Present study was conducted to investigate the pain and hematological effects of disbudding by EG. So, the cortisol serum level, blood biochemical parameters, and some vital organ enzymes in the goat kids were compared following two methods of disbudding: eugenol and cautery.

Results

1. ***Clinical Evaluation***

1.1. Body Temperature: There was a relationship between changes in rectal temperature and time (Figure 1). There was no statistically difference in rectal temperature between group 1 and 3 ($P>0.05$).

1.2. Pulse Rate: There was no relationship between experimental period and pulse rate. (Figure 2).

1.3. Respiratory Rate: There was a significant change between baseline and group 1 immediately after disbudding (Figure 3, $P\leq0.05$).

2. ***Serum Cortisol Level Evaluation:*** The time interaction was measured for cortisol level (Figure 4). There was a difference between baseline and immediately after disbudding in groups 2 and 4 ($P\leq0.05$). Also, mean level of cortisol, in both groups was decreased after 30 min, but there was no significant difference in the serum cortisol level between groups until 120 min after disbudding ($P>0.10$).

3. CBC, Blood Chemical, and Vital Organ Serum Enzyme Evaluation: The results of CBC test in group 5 showed a decrease in the number of red blood cells, hemoglobin, hematocrit percentage, and platelet compared to those obtained 24hr before and after disbudding by EG (Table 2; $P>0.05$). White blood cell count was not significantly different 24hr after the injection of EG.

Discussion

This evaluation was done through the measurement of serum cortisol level and TPR to determine the pain and stress levels in disbudding by cautery compared to the EG injection over time. Analysis of the cortisol level in disbudding by cautery showed more pain inducing than EG administration in goat kids for 30 min after disbudding. This result was also supported by the TPR measurement, in other words, at early hours after disbudding by cautery, more physiological changes occurred due to stress compared to our new disbudding alternative method. Similarly, other studies have reported that disbudding by cautery was more painful than the clove oil injection,[26] while requires additional analgesia or anesthesia.[27,28] Furthermore, the increase of approximately 3μg/dL blood cortisol in groups 4 and 2 (Figure 1) combined with an increase in the body physiological activity in groups 1 and 3 (Figure 2-4) indicates a physiological response to the pain and stress stimulus, however, this increase in the groups disbudded by EG was less than those disbudded by the cautery, indicating low levels of stress and pain in EG injection.

Pain and stress through sympathetic effects increase changes in the heart and respiratory system and reduce peripheral blood flow. Stress and pain also affect the appetite and nutrition of animals, and subsequently, can result in weight loss in food animals.[29] The reaction of both sympathetic and HPA axis under stress conditions is well-known.[30] In the meantime, the response of the sympathetic axis to the stress stimulus is very rapid and it releases the catecholamines into the blood serum. Cortisol serum level has been used to measure pain and stress in ventricular resection surgery, sterilization, and pain induced by electric shock.[31-34] Cortisol levels change throughout the day, so, intermittent blood sampling should be used to evaluate this parameter more accurately.[18] To determine the effects of stress and pain on body, in addition to cortisol level, measurement of vital parameters (TPR) would be also helpful.

EG, as the main ingredient of clove oil, has anti-inflammatory and analgesic effects that are routinely used in dentistry (0.1% solution). Therefore, it can be concluded that the reduction in pain following eugenol injection may be a result. However, in some studies, this analgesia has not been clearly shown, or in some cases, the amount of pain caused, has been calculated as the same with cautery.[26,35] According to our results, the amount of pain due to EG injection was significantly less than the cautery, especially immediately after injection, and 240 - 480 minutes later. As our study was intended to compare the values of serum cortisol levels in the two groups of eugenol and cautery with the baseline values, perhaps if there was an intact goat kids group as sham for cortisol measurements, we could better show the difference in pain between these methods. The reason of pain caused by eugenol injection, which is clearly observed in the results of both our clinical and serum methods, is probably caused by the type of use (injection by needle) and also the volume of fluid entering the subcutaneous area. The phenolics structures can also cause local irritation.

Mc Meekan et al., (1998), compared cortisol serum levels in dehorning of cattle by local anesthetics alone or with non-steroidal anti-inflammatory drugs (NSAIDs). They found that cortisol serum level in group that received local anesthetics and NSAID was lower than those received local anesthetics alone. This study clearly showed that the disbudding is completely painful operation and analgesic requirement to relief the pain and the stress is inevitable.[36]

In a similar study conducted by Stilwell et al. (2010), similar results about disbudding of cattle by cautery were obtained. Also, it was revealed that Xylazine as a sedative cannot prevent the increase of cortisol serum levels following dehorning of cattle by cautery, while there was no significant difference in the cortisol level between the animals received Xylazine and those received no Xylazine, therefore, it had no acceptable effects on the pain reduction in cattle dehorned by cautery.[37] although in another study by Stilwell et al. (2012), Carprofen was able to keep the cortisol serum level low by controlling the pain following cautery dehorning of cattle.[38] Stilwell et al. (2009) showed that the application of a local anesthetic method with NSAIDs in cautery dehorning of cattle, could reduce the pain during and after dehorning.[39]

Based on previous studies, the method of using caustic paste disbudding in calves and goat kids is more painful than other routine methods. In this method, the serum cortisol level was in high range compared with other groups done by cautery and clove oil (194 nmol/L by caustic paste vs. nmol/L by clove oil). Also, the behavioral changes related to stress level were clearly observed and showed similar overlap with changes in serum cortisol levels. However, the use of analgesics such as alpha 2 agonists (Xylazine) in calves can reverse the situation in favor of the caustic paste method, which can be due to the lack of behavioral symptoms similar to those in animals under painful procedures.[26]

Bengtsson et al., (1996) and Hempstead et al. (2018), both were of the opinion that the use of cryosurgery for disbudding is more painful than the cautery. Although, according to the authors, the interpretation of behavioral patterns in different species may have conflicting results, so measurements of the serum cortisol levels can help provide a reasoned rationale.[40,27]

Body temperature changes can be an important parameter in measuring the pain or inflammation. Temperature changes seen in the results of the current study, along with others, show a low level of pain in the method of dehydration by EG. Although it is possible, due to the low number of animals in each group because of ethical reasons, as well as individual variation in the tested herd, the values obtained are not statistically significant differences.

The increasing number of white blood cells in blood samples of group 5, 24hr after disbudding by the EG injection compared to 24hr before it, was not indicative of leukocytosis because it was limited to the normal range of white blood cells. The increase of platelet count was in normal range and it may have been due to the EG injection in horn buds.

Since EG leads to hepatic metastasis, based on some studies, the systemic use of this compound may cause toxic hepatitis. Thompson et al. (1991) investigated the toxicity and metabolism of EG with the concentration of 1 mM in the rat hepatocyte cell line. They revealed that EG is metabolized in hepatocytes and Quinone-Methide is a probable cause of toxic hepatitis by EG.[41] However, in this study, the results of serum enzymes measurement, especially AST, SDH, and GGT, showed that toxic hepatitis was not induced due to the SC injection of 0.1 ml EG in horn buds. Although AST increased 24hr after the EG injection due to hemolysis or liver injury, but since the SDH was within normal range, the possibility of hepatitis could be ruled out.

Conclusions

Interpretation of physiological parameters along with behavioral symptoms during the disbudding or other painful procedure can help to achieve better results. But due to the difference between animal species seems it’s impossible to suggest the same pattern for all under various painful procedures. However, such studies about the routine procedures will increase the animal welfare by reducing the pain, which itself promises a better future in animal husbandry.

In general, disbudding by EG compared with cautery, induces less stress and pain in the goat kids and has no harmful effect on the other vital organs of the body. Therefore, this method can be used or suggested as a suitable alternative.

Methods

This study was carried out on 25, five-day-old, male and healthy Raieni (Cashmere) goat kids. The animals used in this study were acquired from the domestic animal farm of Shahid Bahonar University of Kerman. All kids received colostrum at birth and to minimize potential stress, they were not separated from their dam or other kids. Kids were kept in the research farm in standard kids pen with clean and dry bedding during the study (March to April). They feed with their mother's milks two times in the day under the farm routine standards plan. Kids had access to fresh water in a pond attached to the pen walls. The temperature ranged between 10-25°C (day and night) and the humidity was 21% in the barn. The animals randomize divided kids were ear tagging and the number of all 25 registered. To separate the stress effect of blood sampling with disbudding, as well as to avoid hypovolemic shock due to intermittent blood sampling in goat kids, the animals were randomly allocated to five equal groups (n=5). In 1 and 2 groups, disbudding was done by electrical cautery (HORN'UP ®), specifically designed for goat kids. In group 3 to 5, disbudding was performed by SC injection of 0.1 ml EG (Table 1). Subcutaneous administration of eugenol was performed by insulin syringe in the horn bud region. For this purpose, a 0.5-inch 30-gauge needle was used. The needle was penetrated lateral aspect and guided under horn bud.

In the end of the study, all the animals were received clinical examination by farm veterinarian and they were returned to their flock.

Clinical Evaluation: Same as above reason, the TPR evaluation groups were isolated from serum cortisol level evaluation groups to separate stress effect of clinical evaluation and disbudding. In 1 and 3 groups, the animals were kept in the goat kid special box; the body temperature measured by a digital rectal thermometer, and the pulse rate and respiratory rate measured by a stethoscope (Riester Duplex 4200, Germany). All parameters evaluated before disbudding (baseline), immediately after disbudding, and every two hours until 8hr, and 24hr following disbudding by cautery or EG administration.

Serum Cortisol Level Evaluation: Serum cortisol level measured in 2mL blood samples that collected by jugular vein catheterization, before (baseline), immediately after disbudding, and 30, 60, 120, 240, 480min post disbudding in 2 and 4 groups. The jugular vein catheterization was placed 12hr before disbudding. For this aim, the animals were kept in goat box while sampling performed. The jugular vein catheterization was placed 12hr before disbudding. The blood samples transmitted to micro tubes and they were centrifuged at 4000 rpm for 10 min, and then, separated serums were stored at −80°C until analysis. Serum cortisol level measured by enzyme-linked immunosorbent assay (ELISA) commercial cortisol kit (Monobind, USA) in commercial veterinary laboratory. The operators were blind to the treatment groups.

CBC, Blood Chemical, and Vital Organ Serum Enzyme Evaluation: In group 5, blood samples (5.5 mL) were taken from jugular vein, 24hr before (baseline) and 24hr after EG administration without catheterization. The blood samples were poured into two different tubes (2.5 mL in the CBC tube with EDTA and 3 mL in the serum separator tube) (FL medical, Italy) to test CBC and blood enzymes. The CBC test was done by commercial auto analyzer. To perform blood chemistry test, the blood samples were centrifuged at 4000 rpm for 10 min, and then, the serum was separated and stored at −20°C until analysis. The enzymes included aspartate transaminase (AST), alanine transaminase (ALT), gamma-glutamyl transpeptidase (GGT), succinate dehydrogenase (SDH), and alkaline phosphatase (ALP) as the liver enzymes, and creatine phosphokinase (CPK) as the muscles or heart muscles enzyme. All these enzymatic tests were performed using enzymatic colorimetric method (Pars Azmoon commercial kits, Iran) and biochemistry autoanalyzer (Alpha Classic, Sanjesh Co., Iran). Other biochemical parameters were total protein (TP), blood urea nitrogen (BUN), and creatinine (Cr, Jaffe method; Pars Azmoon commercial kits, Iran, and biochemical auto analyzer; Alpha Classic, Sanjesh Co., Iran). Both analyses were done using standard method in commercial veterinary laboratory. The operators were blind to the treatment group, too.

All data were presented expressed as mean ± standard error. Statistical analysis was performed using repeated measures analysis of variance (RMANOVA). For running the statistical analysis, we considered treatment as the fixed and time as the random effects. Before doing the analysis, we checked the data for normality using the Kolmogorov-Smirnov test. Also, data were checked for the presence of outliers. As the data were normal, no transformation was needed. Statistically significant level was considered at P<0.05 and statistical analysis was done using SPSS software (SPSS Inc. Released 2011. SPSS for Windows, Version 20, IBM, SPSS Inc.).

All procedures were approved by the Animal Ethics Committee of Shahid Bahonar University of Kerman (Ethical code: IR.UK.REC.1398.001).

Abbreviations

EG: Eugenol; TPR: temperature, pulse, and respiratory rate; HPA: the hypothalamic-pituitary-adrenal axis; SC: subcutaneously; NSAIDs: non-steroidal anti-inflammatory drugs; AST: aspartate transaminase; ALT: alanine transaminase; GGT: gamma-glutamyl transpeptidase; SDH: succinate dehydrogenase; ALP: alkaline phosphatase; CPK: creatine phosphokinase; TP: total protein; BUN: blood urea nitrogen; Cr: creatinine.

Declarations

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Not applicable

Authors' contributions

MMM designed all the experiments. MFA performed the all experiments until sample collection. HS performed the statistic analyses. MFA, MMM and MMO wrote the manuscript. All authors read and approved the final manuscript.

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Competing interests

The authors decelerate there was no competing interest in their research.

Availability of data and materials

The datasets during and/or analyzed during the current study available from the or responding author on reasonable request.

Consent for publication

Not applicable

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Tables

Tab. 1: The names of the groups involved in the study based on the parameters measured.

	Clinical Parameters (TPR)	Serum Cortisol Level	CBC & Blood Chemistry
Cautery groups+	1	2	-
Eugenol groups+	3	4	5

(n=5)

Tab. 2: CBC and biochemical evaluation of goat kids disbudded by SC injection of eugenol (0.1 mL; group 5, mean ± SE).

CBC Panel	Group 5			normal range[1]	Biochemical Panel	Group 5			normal range
	24h before	24h after				24h before	24h after		
RBC* ($\times 10^6$/ µL)	6.22	7.326	↑	8.0-18.0	BUN (mg/dL)	42.4	30.6	↓	
Hb (g/dL)	7.66	8.52	↑	8.0-12.0	Cr. (mg/dL)	0.538	0.496	↓	
HCT* (%)	20.62	24.6	↑	22-38	ALT (U/L)	18.00	19.00	↓	15.3-52.3
MCV (fL)	34.36	33.06	↓	16-25	AST (U/L) *****	68.00	91.80	↑	167-513
MCH* (g/dL)	12.3	11.9	↓	5.2-8.0	GGT***** U/L	117.60	76.00	↓	20-56
MCHC* (g/dL)	37.2	35.08	↓	30.0-36.0	ALP U/L	110.80	180.20	↑	93-387
WBC* 1000/Cumm	9.424	8.474	↓	4-13	CK U/L	96.40	83.80	↓	0.8-8.9
PLT* 1000/ µL	162.22	66.68	↓	100-300	SDH U/L	15.5	16.1	↑	14-23.6

(*) show significant difference ($p<0.05$) in comparison with 24hour before, and after disbudding by EG injection.

(↓) increase or (↑) decrease in measurement parameters.

1- Ettinger, 2017

Figures

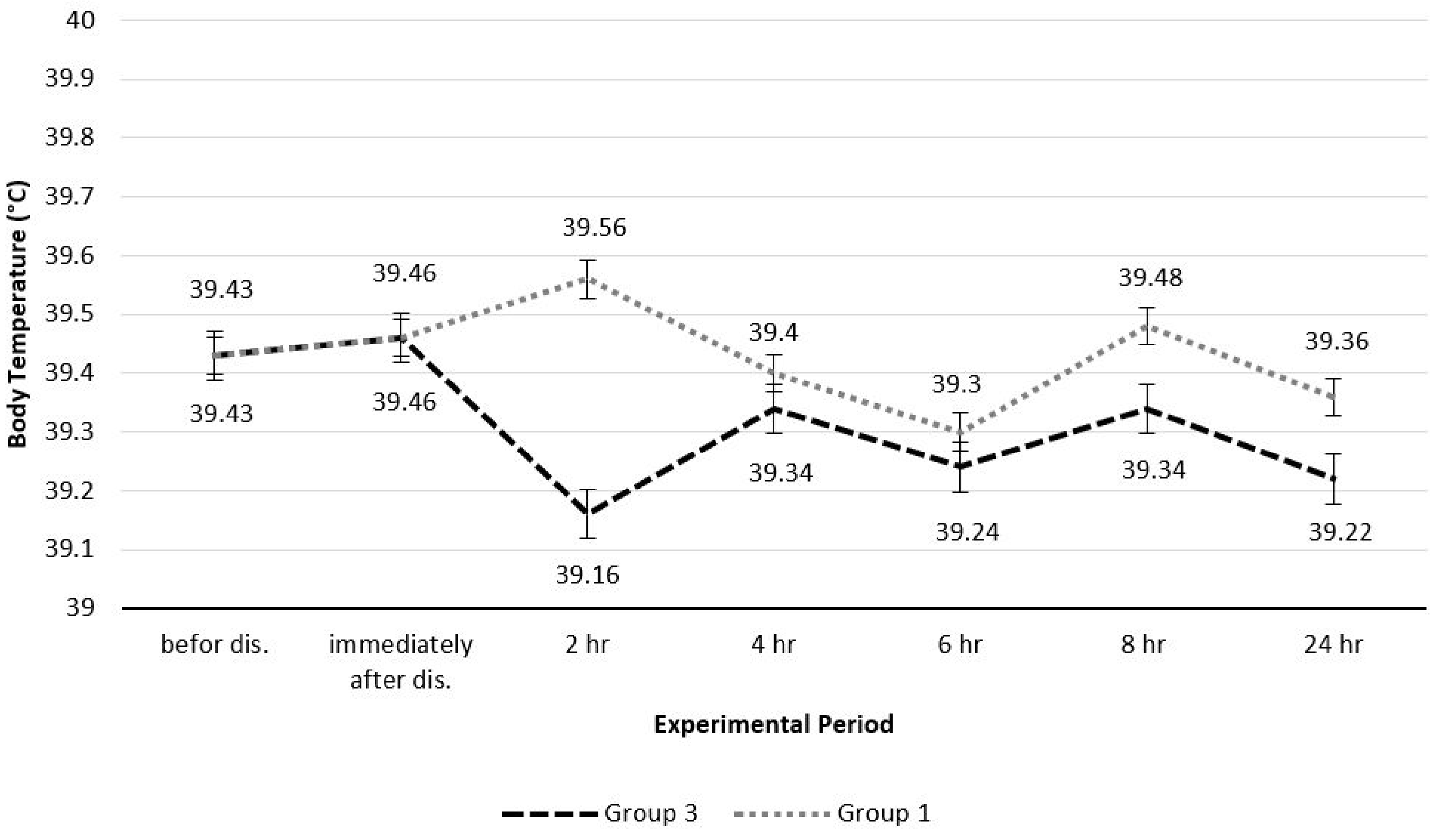

Figure 1

Body temperature (°C) of goat kids's changes in group 1 and 3(cautery) compared to those in baseline 2 hr after disbudding.

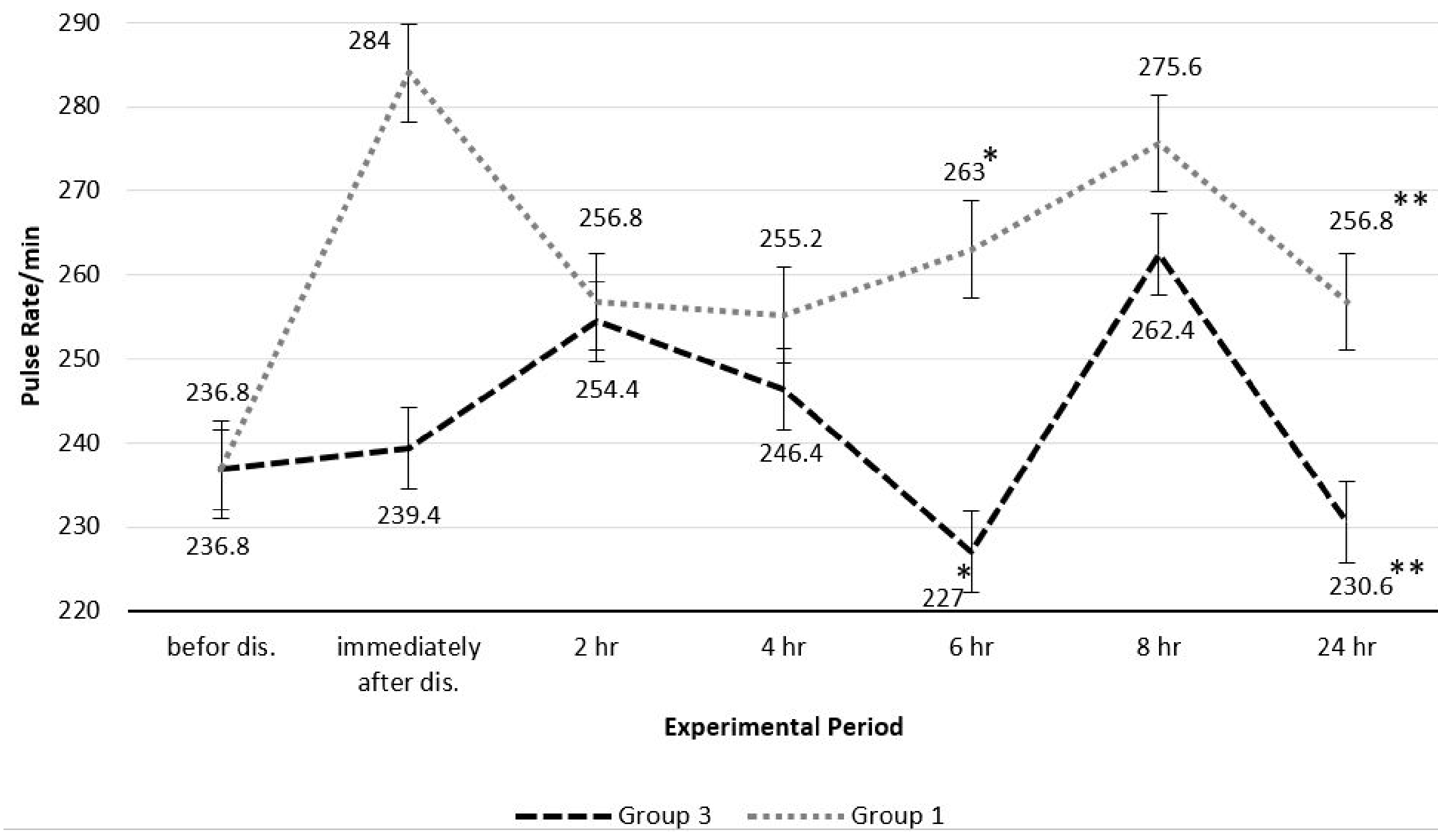

Figure 2

Changes in pulse rate (n/min) following disbudding by cautery or SC injection of eogenol (0.1 mL) in groups 1 and 3, respectively. *,** significant difference in results between different groups (p≤0.05)

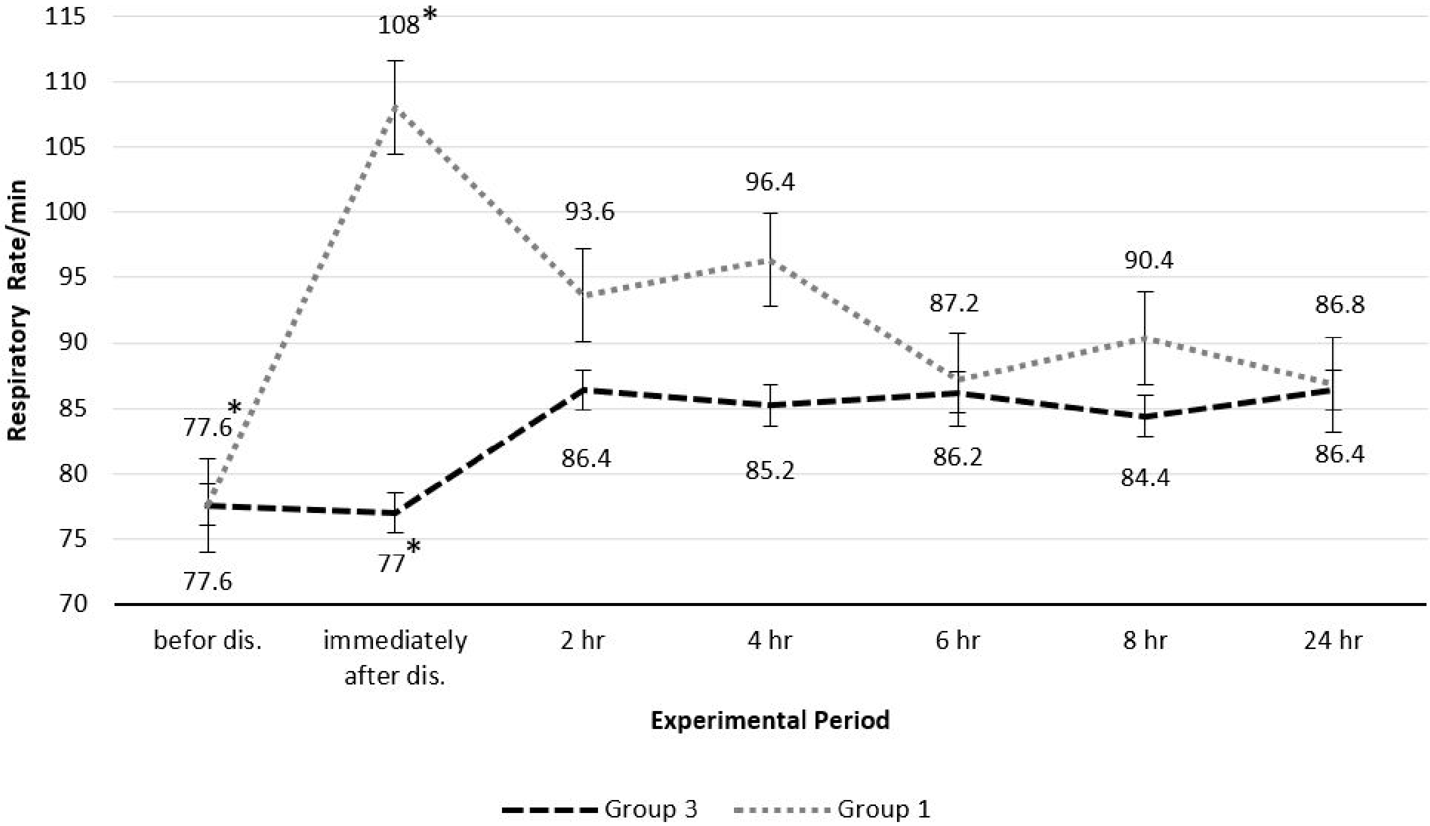

Figure 3

Effect of disbudding on respiratory rate (n/min) using cautery or SC injection of eogenol (0.1 mL) in groups 1 and 3, respectively. * significant difference in results between different groups (p≤0.05)

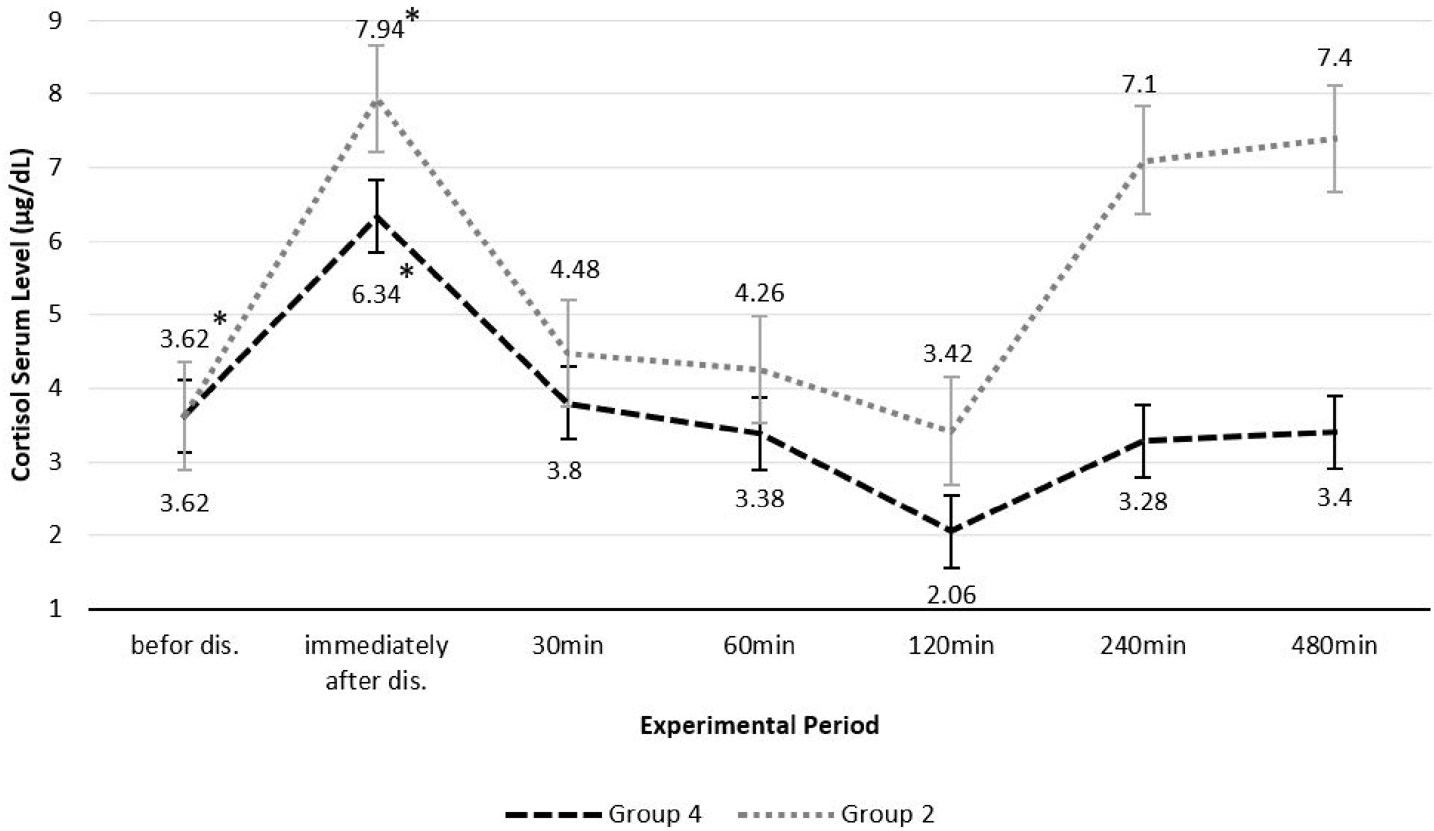

Figure 4

Serum cortisol level (mg/dL; mean ± standard error) before (baseline) and immediately after disbudding, also, over 30, 60, 120, 240, and 480 min; by cautery or SC injection of eogenol (0.1 mL) in groups 2 and 4, respectively. * significant difference in results between different groups ($p \leq 0.05$)

Supplementary Files

This is a list of supplementary files associated with this preprint. Click to download.

- AuthorChecklistFull.pdf
- Graph.docx