

Clove oil and cautery disbudding efficacy

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4 The effectiveness of clove oil and cautery disbudding methods on preventing 5 horn growth in dairy goat kids

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21 **Abstract**

22 The effectiveness of clove oil and cautery disbudding on horn growth was evaluated in goat
23 kids. The study used 243 Saanen doe kids (4 ± 1.0 days old; mean \pm SD) on two commercial
24 dairy goat farms, and were disbudded with either (i) clove oil injection (CLOVE), (ii) a cautery
25 iron and bud removed (BUDOFF), or (iii) a cautery iron with bud left intact (BUDON). Each
26 kid received a different treatment per bud, which were balanced between buds (left and right)
27 and randomly allocated. A trained observer monitored bud growth following treatment for 3
28 months recording either: N: no growth, H: normal horn, S: abnormal horn (scur), or SC: soft,
29 fibrous lump (scorn). After the final observation, buds were assessed for the probability of
30 detecting (i) success (no growth), (ii) scurs, (iii) horns or (iv) scorns [with 95% CI]. The
31 probability of success for BUDOFF (0.77 [0.63, 0.87]) was higher than for BUDON (0.20
32 [0.11, 0.34]) and CLOVE (0.09 [0.04, 0.18]; $P \leq 0.05$). Furthermore, the probability of success
33 for BUDON was higher than for CLOVE ($P \leq 0.05$). The probability of scurs was higher for
34 CLOVE (0.72 [0.63, 0.80]) than BUDOFF (0.25 [0.17, 0.34]) and BUDON (0.30 [0.21, 0.39];
35 $P \leq 0.05$). There was no difference in the probability of scurs for BUDOFF and BUDON ($P >$
36 0.05). The probability of horns was higher for CLOVE (0.21 [0.15, 0.29]) than BUDON (0.02
37 [0.01, 0.06]; $P \leq 0.05$); horns were not observed for BUDOFF. The probability of scorns for
38 BUDON, the only treatment that led to scorns, was 0.41 (0.25, 0.60). These results suggest that
39 BUDOFF was more effective at preventing growth than CLOVE and BUDON and appears the
40 most effective method, of the methods tested, for disbudding kids. Future research should
41 explore different clove oil administration methods or other alternatives to cautery disbudding
42 that may be both efficacious and cause less pain.

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45 **Keywords:** Dairy goat, kid, efficacy, horn bud, horn growth, scur, clove oil, cautery disbudding.

46 **Introduction**

47 Dairy goat kids are routinely disbudded, usually within the first week of life [1]; the
48 practice involves the destruction of the horn buds to prevent horn growth. The predominant
49 method involves using a hot cauterly iron to cauterise and remove horn buds. Disbudding is
50 carried out to reduce the risk of injury to other goats [2] and their human handlers, and allows
51 for more space at the feed rail or in lying areas [3]. Current disbudding methods for goat kids
52 have been adapted from those used for calves, such as cauterly disbudding, caustic agents and
53 surgical methods [4-6].

54 It is generally accepted that cauterly disbudding causes pain and distress in goat kids as
55 evidenced by intense and frequent vocalisations, leg shakes during the procedure [7], elevated
56 plasma cortisol concentrations and increased frequencies of head shaking, rubbing and
57 scratching post-disbudding [8-10]. Furthermore, potential complications associated with
58 cauterly disbudding include second- or third-degree burns, inflammation, thermal injury to the
59 skull and brain (causing necrosis), infection and an increased risk of mortality [11-13].

60 If disbudding of goat kids is not performed, then adult goats can be dehorned; this
61 involves surgical removal of the horns, which creates an opening into the frontal sinus and
62 causes more pain than disbudding [5]. It can also lead to complications such as prolonged
63 healing, discharge/infection, inflammation, regrowth of horns, dehiscence or even death [14,
64 15]. Therefore, disbudding is the preferred method for preventing horn growth.

65 There is limited research on the efficacy of different disbudding methods (including
66 cauterly disbudding) on horn or scur growth in goat kids or calves. Disbudding is considered
67 successful if no horns or scurs grow (i.e. all horn bud tissue is destroyed). Scurs can be defined
68 as distorted horn regrowth following disbudding [15]. Problems associated with scurs include
69 being aesthetically unpleasing, breaking off easily causing open wounds and increasing the risk
70 of infection and potential abnormal growth back towards the animal's head, requiring surgical

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71 removal. Cautery disbudding can be performed by either (i) totally removing the ring of tissue
72 containing the horn bud cells or (ii) cutting/burning a circular ring around the horn bud but
73 leaving it intact. It is unclear which method of cautery disbudding is most effective in
74 preventing scurs or horns.

75 A recent study [9] used the physiological and behavioural responses of dairy goat kids
76 to evaluate alternatives to cautery disbudding (i.e. caustic paste and cryosurgical disbudding,
77 and clove oil injection). Clove oil injection elicited a similar cortisol response and number of
78 head shakes and scratches as cautery disbudding, indicating a similar experience of pain [9].
79 Even though the pain response to clove oil injection appeared to be similar to that generated
80 by the cautery iron, the clove oil method caused less tissue damage [9]. Consequently, clove
81 oil injection may result in faster healing times and lower rates of infection or skull or brain
82 injury. Caustic paste and cryosurgical disbudding appeared to cause more pain than cautery
83 disbudding [9], and therefore were not included as treatments in the present study.

84 Clove oil, which was traditionally used in dentistry as a topical analgesic and antiseptic
85 [16], contains a high concentration of eugenol (i.e. 80-85%), which has been shown to cause
86 cellular necrosis (and inflammation) in the oral mucosa of rats [17]; it can also be highly
87 cytotoxic for human skin cells [18]. Recently, clove oil has been shown to cause local cellular
88 necrosis of horn bud tissue resulting in arrested horn growth in calves [19] and goat kids [20].
89 Further research is required to evaluate the effect of clove oil on horn and scur growth in goat
90 kids.

91 The objective of this study was to evaluate the effectiveness of clove oil injection and
92 two methods of cautery disbudding (i.e. horn buds removed vs. left intact) on horn bud growth
93 in dairy goat kids. We predicted that clove oil injection would result in similar levels of horn
94 and scur growth as cautery disbudding, based on the success rates (100%) reported by others
95 [20]. We also predicted that cautery disbudding with horn buds removed, would result in less

96 horn and scur growth than leaving the horn buds intact due to increased potential for complete
97 cell destruction.

98

99 **Materials and methods**

100 The Ruakura Animal Ethics Committee approved the use of animals prior to the
101 commencement of the study (Protocol No. 14213).

102

103 **Pilot study**

104 A pilot experiment was carried out to ensure that administration of clove oil into the
105 horn bud had no detrimental effect on the skull and brain of goat kids before further studies
106 were conducted. On a commercial dairy goat farm in the Waikato region of New Zealand, 10
107 Saanen doe kids were selected from unwanted stock based on age (i.e. 2-3 days old) in June
108 2017. The animals were injected with 0.2 mL of clove oil into each horn bud using the
109 procedure described in Hempstead et al. [9]. Kids were reared in a small barn separate from
110 the farmer's replacement stock and fed using a 10 L bucket feeder with 6 teats. Two weeks
111 after clove oil injection, 5 kids were euthanized by a veterinarian. The remaining 5 kids were
112 euthanized 1 week later (i.e. 3 weeks after clove oil injection) to assess the effects of clove oil
113 over time. After euthanasia, the bodies were transported to a post mortem facility at the
114 Ruakura Research Centre, in Hamilton, New Zealand, and gross examination was then
115 performed by a veterinary pathologist.

116 Firstly, the skin over the head was visually assessed and then the skin was removed so
117 that the outside of the skull could be examined. Next, the head was cut transversely, just caudal
118 to the horn buds using a commercial meat band saw. The brain was removed from the front
119 part of the skull and the inner surface was examined for evidence of damage (e.g., perforation,

120 hyperaemia) or inflammation beneath the horn bud sites. The dorsal surfaces of cerebral
121 hemispheres beneath these sites were examined for ulcerations.

122 Large black scabs covered the horn buds 2 weeks post-injection of clove oil. There were
123 localised dark patches on the skull below the horn buds for the 5 kids euthanized 2 weeks after
124 treatment, with no evidence of damage on the inside of the skull or the brain. At 3 weeks post-
125 injection, patches of newly healed skin were observed as well as scurs in 3 out of the 5 kids.
126 Discolouration of the skull beneath the horn buds was apparent in only one kid. There was no
127 evidence of inflammation, perforation or infection in the skull, meninges or brain associated
128 with the clove oil injection for any of the kids.

129

130 **Animals and housing**

131 This study was conducted on two private commercial dairy goat farms within the
132 Waikato region in New Zealand between June and December 2017. A total of 243 Saanen doe
133 kids (4 ± 1.0 days old; mean \pm SD) were used (Farm A: 189 kids; Farm B: 54 kids) and were
134 selected for inclusion based on age (2 – 6 days old) and size of horn buds (< 16 mm). Kids had
135 an average body weight of 4.0 ± 0.55 kg (range: 2.7 – 6.1 kg). Kids were housed in pens (3.5
136 x 2.0 m) with approximately 15 kids/pen until they were 2 weeks old or were feeding
137 independently, at which point they were moved to larger pens (9.0 x 5.0 m) with approximately
138 45 kids/pen.

139 The kids were reared as per routine practice on each farm [21]. The ground within the
140 pens was covered with untreated pine shavings (approximately 10 cm deep). Each pen had *ad*
141 *libitum* access to milk replacer (SprayFo, AgriVantage, Hamilton, New Zealand; mixed
142 according to packet instructions) in feeders (Milk Bar, Waipu, New Zealand) with 10
143 teats/feeder (36 L capacity) and fresh water in a trough.

144

145 **Experimental design**

146 A power analysis for a binary outcome bio-equivalence trial was conducted based on
147 80% power, a 5% significance level and an equivalence range of +/- 0.15. We used a
148 randomized split-plot design with a different treatment per horn bud, with treatment day, kid,
149 and farm as blocking variables. A replicate consisted of all pairs of treatments and whole
150 replicates were completed per treatment day. Horn buds were randomly allocated treatments
151 ($n = 162$ horn buds/treatment) balanced for kid age. Kids were given a coloured collar for
152 treatment identification within the pens as they were housed with others not experiencing any
153 treatment. The same veterinarian performed all treatments. The experiment was conducted on
154 8 treatment days over a 2.5-week period. Kids were fed approximately an hour before treatment
155 and then taken (one at a time) from their home pen and placed in a restraint device (described
156 in Hempstead et al. [10]). Treatments were performed in an alleyway alongside the home pens
157 and treatment order was randomly assigned. Hair covering the horn buds was removed with an
158 electric clipper (Laube, 505 cordless kit, Shoof, Cambridge, New Zealand) to expose the horn
159 buds. Prior to treatment, kids received an oral non-steroidal anti-inflammatory drug (Loxicom
160 0.5 mg/mL oral suspension for dogs, Norbrook Laboratories Ltd, Newry, UK; 0.2 mg/kg BW)
161 and a cornual nerve block using lidocaine (Lopaine 2%, 20 mg/mL, Ethical Agents, Auckland,
162 New Zealand; 0.1 mL/horn bud) to reduce pain associated with treatment.

163 Treatments included:

164 (i) BUDOFF: Disbudding using a cautery iron (“Quality” electric debudder, 230 V, 190 W;
165 Lister GmbH, Lüdenscheid, Germany), which was heated for 20 min (to reach c. 600°C)
166 prior to being pressed to each horn bud for a total of 5.9 ± 1.09 s (mean \pm SD). Horn buds
167 were then removed by pressing the iron down and rotating so the skin was cut and the buds
168 forcibly flicked out, as described in Hempstead et al. [8].

169 (ii) BUDON: The same procedure as for BUDOFF, except that the horn buds were cut but not
170 removed. The iron was held to each horn bud for a total of 4.8 ± 1.08 s on average.

171 (iii) CLOVE: Clove oil (C8392, 100mL, 83-85% eugenol, Sigma-Aldrich, Saint Louis, MO)
172 was injected (0.2 mL; [20]) laterally into the centre of each horn bud at a 45° angle between
173 the ear and muzzle (20.9 ± 8.39 s; mean completion time \pm SD); details of the procedure
174 are described in Hempstead et al. [9].

175 After treatment, BUDON and BUDOFF wounds were sprayed with antibacterial spray
176 (Tetravet, Bayer New Zealand Ltd., Auckland, New Zealand) to prevent infection. Horn buds
177 treated with CLOVE did not receive spray as there were no open wounds. Kids were then
178 returned to their home pen and were monitored for 2 h post-treatment to ensure no
179 complications associated with treatments occurred. The health status and horn bud growth of
180 the goat kids was assessed for 5 months post-treatment. Any kids that died over the course of
181 the experiment were examined post-mortem by a veterinarian to determine cause of death.

182

183 **Horn bud growth categories**

184 Horn bud growth categories were defined before the start of the experiment. Each horn
185 bud was categorised based on whether it displayed normal horn growth (H), abnormal growth
186 or scurs (S) or no evidence of growth (N; Fig 1). An extra category was added after the first
187 farm visit 2 weeks after treatment as there were growths that could not be categorised as either
188 H or S – a scorn (SC; Fig 1). A horn was defined as having normal growth without
189 abnormalities. A scur was defined as any abnormal growth with a hardened (keratinised)
190 surface that could be felt by hand in the horn bud area. A scorn was defined as a soft and fibrous
191 (observed when cut) growth with a wide base and usually a rounded tip. N was recorded when
192 the skin was smooth and there was no horn growth; this was considered a success. Horn buds
193 were categorised into the four groups fortnightly for 2 months and then monthly for a further

194 3 months by a trained observer. The observer remained blind to the treatments each kid
195 received. Once growth was observed, that treatment was considered unsuccessful and if both
196 horn buds had evidence of growth, the animal was no longer monitored in subsequent checks.
197 The probability of success, scurs, horns or scorns for each treatment at the final observation
198 are presented.

199

200 **Fig 1. Categories of horn growth in dairy goat kids.**

201 No growth = N, scur = S, horn = H and scorn = SC. Goat kids were disbudded using either a
202 clove oil injection (CLOVE), a cautery iron with the horn buds removed (BUDOFF) or a
203 cautery iron with the horn buds left intact (BUDON).

204

205 **Statistical analysis**

206 Genstat statistical software (version 18, VSN International, Hemel Hempstead, UK)
207 was used to analyse the data. The binary response variables used for analyses included success
208 (no growth = N), scurs (growth = S), horns (growth = H) and scorns (growth = SC); each
209 response variable was assumed to be binomially distributed. Analysis of the differences
210 between treatments were performed independently for all response variables. In addition, a bio-
211 equivalence analysis was performed for the probability of success, with 80% power and an
212 equivalence range of +/- 0.15. Bio-equivalence was assessed for each treatment with BUDOFF
213 (considered the reference treatment) and also for CLOVE and BUDON (with BUDON as the
214 reference treatment). We used a generalised linear mixed model for the analyses with a logit
215 link. The fixed effects were for treatment and the treatment on the other horn bud (i.e. of the
216 same kid). The random effects were for kid, farm, horn bud (left or right) and treatment date
217 within farm. Differences between treatment means were compared using Fisher's protected
218 least significant difference test at the 5% significance level.

219

220 **Results**

221 Of the 243 kids were enrolled in the study, 12 died before their 24 horn buds could be
222 assessed 2 weeks following treatment; one animal (BUDON/BUDOFF) died 2 weeks post-
223 treatment as a result of meningitis below the horn bud but the others died from complications
224 not related to treatment (i.e. pneumonia, digestion issues). Data was missing for 9 BUDON and
225 BUDOFF horn buds and 6 CLOVE horn buds. The differences in the probabilities of success,
226 scurs, horns or scorns are presented in Fig 2. There was an effect of treatment on the probability
227 of success ($F_{2,443} = 43.3, P < 0.001$). The probability of success for BUDOFF horn buds was
228 higher than for BUDON and CLOVE horn buds ($P \leq 0.05$). Furthermore, the probability of
229 success for BUDON horn buds was higher than that of CLOVE horn buds ($P \leq 0.05$). There
230 was no evidence of bio-equivalence for the probability of success between BUDOFF and
231 CLOVE, nor between BUDOFF and BUDON; however, the probabilities of success for
232 BUDON and CLOVE horn buds were bio-equivalent.

233

234 **Fig 2. Probability of the four categories of horn growth with 95% confidence intervals for**
235 **goat kids disbudded using three different techniques.**

236 (A) Success (no growth = N), (B) scurs (growth = S), (C) horns (growth = H) and (D) scorns
237 and horn combined (growth = SC or H). Goat kids were disbudded using either clove oil
238 injection (CLOVE; n = 156 horn buds), a cauterization iron with the horn buds removed (BUDOFF;
239 n = 153 horn buds) or a cauterization iron with the horn buds left intact (BUDON; n = 153 horn
240 buds). Means with differing subscripts are significantly different at $P \leq 0.05$.

241

242 There was a treatment effect on the probability of developing scurs ($F_{2,452} = 28.3, P <$
243 0.001). The probability of scurs was higher on CLOVE than BUDOFF and BUDON horn buds

244 ($P \leq 0.05$). There was no difference in the probability of scurs for BUDOFF and BUDON horn
245 buds ($P > 0.05$).

246 There was a treatment effect for the probability of horns ($F_2 = 8.9$ [chi-square test used
247 as denominator degrees of freedom (ddf) were not estimable], $P < 0.001$). The probability of
248 horns was higher for CLOVE than BUDON horn buds ($P \leq 0.05$); horns were not observed for
249 BUDOFF horn buds.

250 Scorns were only observed for BUDON horn buds (0.41 [0.25, 0.60]). There was an
251 effect of treatment on the probability of horns and scorns combined ($F_2 = 11.2$ [chi-square test
252 used as ddf not estimable], $P < 0.001$). The probability of scorns and horns was higher for
253 BUDON than CLOVE and BUDOFF horn buds ($P \leq 0.05$).

254

255 **Discussion**

256 The effectiveness of clove oil injection and two methods of cautery disbudding (i.e.
257 horn buds removed vs. left intact) in preventing horn growth were evaluated in dairy goat kids.
258 Clove oil injection has been previously reported by Molaei et al. [20], to be 100% successful
259 in preventing horn growth in kids as well as calves [19]. In the present study, the CLOVE
260 treatment appeared to be less effective at preventing horn growth than either of the cautery
261 disbudding methods based on the high incidence of scurs and horns. Clove oil injection is a
262 novel method of disbudding for goat kids compared with cautery disbudding (adapted from use
263 in calves), which is the most commonly used method for disbudding goat kids worldwide [1].
264 Higher proportions of scurs on horn buds treated with clove oil compared with cautery
265 disbudding may be associated with difficulties in restricting movement of the head during
266 treatment. Clove oil was applied using a needle injected laterally into the buds whereas cautery
267 disbudding involved pressing an ergonomic cautery iron down on the head. Consistent
268 administration of the full volume of clove oil to the correct location (centre of the horn bud)

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269 was not always possible. The injection is likely to cause discomfort or pain [9] and kids
270 generally struggled (rapid jerks of the head) during the procedure, resulting in the needle
271 becoming dislodged. The creation of an applicator that can quickly deliver a consistent volume
272 of clove oil to the right location may improve efficacy.

273 Potential explanations for the differences in efficacy of clove oil between Molaei et al.
274 [20] and the present study include differences in methodologies. Molaei et al. [20] used a small
275 sample size (16 vs. 243 kids in the present study), and their own clove oil distilled from the
276 spice (clove oil used in the present study was sourced from a commercial manufacturer). The
277 distilled clove oil appeared to be more effective as it totally prevented horn growth in both doe
278 and buck kids which is notable as horn growth is more precocious in bucks [22]. The exact
279 method of clove oil administration was not completely described in Molaei et al. [20] (e.g., it
280 was not clear how the clove oil was injected or whether the hair was clipped so that any growth
281 could be clearly observed). Perhaps more importantly, Molaei et al. [20] measured horn growth,
282 whereas we evaluated any growth including horns, scurs (of any size) and scorns. It appears as
283 though our methodology for injecting clove oil does not consistently prevent horns or scurs in
284 goat kids and may not be a useful alternative to cautery disbudding with horn buds removed.
285 Interestingly, there was a similar level of success in preventing scurs or horns between CLOVE
286 and BUDON horn buds, indicating that leaving the horn bud intact may also not prevent horn
287 regrowth. The method used in the present study to evaluate horn bud growth may be more
288 comprehensive than methods used by farmers or farm staff and previous studies [19, 20].
289 Future research should examine the exact mechanisms of clove oil action on the horn bud tissue
290 of goat kids.

291 We observed some animals with inflammation of the upper eye lid area below the horn
292 bud associated with the CLOVE treatment. This is interesting, as eugenol, the main component
293 of clove oil, has anti-inflammatory properties [16, 23]. Perhaps blood flow to the horn bud is

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294 reduced due to localised cellular necrosis and the observed swelling was pooling of blood
295 below the horn bud. Measures of tissue sensitivity such as pressure algometry or Von Frey
296 filaments, could be used to evaluate whether this apparent inflammatory response is painful.

297 We also had one kid (treated with BUDOFF and BUDON) die as a result of meningitis,
298 which is likely associated with cauterly iron use. It is well-established that cauterly iron use can
299 cause damage and thermal injury to the skull and brain, resulting in meningitis [11-13].
300 Although only one out of 243 kids died as a result of cauterly disbudding, this demonstrates the
301 capacity for cauterly disbudding to not only cause pain, but mortalities.

302 To the authors' knowledge, this is the first study to quantify the efficacy of cauterly
303 disbudding methods for goat kids. The BUDOFF treatment appeared to be most effective in
304 preventing horns, scurs or scorns compared with the other two methods; this may be due to
305 more complete horn bud destruction as the buds were cauterised and removed. By removing
306 the horn bud, it is easier to ensure that all of the horn bud tissue is destroyed. It is generally
307 considered more efficacious to remove the horn buds than leaving them on [1] and this method
308 has been favoured in other studies [7, 24, 25].

309 The BUDON method resulted in abnormal growths that were soft, fibrous and fitted
310 into neither the horn nor scur categories. The cauterly iron used in the present study had a hollow
311 centre with hot edges that cut into the skin, which may have allowed for the inner cells of the
312 horn bud to continue to grow. This result has implications for farmers and contractors as it
313 suggests that removing the horn buds may be more effective for preventing scurs than burning
314 alone.

315 There is limited information available on horn growth in domesticated farmed goats,
316 therefore comparisons with the literature are difficult. There is however, information on the
317 horn growth of wild adult populations of goats [26], ibex [27] and chamois [28], usually with

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318 respect to environmental effects. Further research on scur or horn growth rates in goat kids
319 after disbudding could help quantify the efficacy of cautery disbudding methods.

320 In order to understand regrowth associated with disbudding, it is important to
321 understand how horns grow. The process involves keratinisation of the horn bud epidermis and
322 ossification of the underlying dermis and hypodermis [29]. Goat horn anatomy is similar to
323 that of cattle horns; the horn comprises tightly packed tubules produced by corium and
324 germinal epithelium, which is attached to the frontal bone. The cornual diverticulum of the
325 frontal sinus forms a cavity within the horn [1]. If the horn bud epidermis is not completely
326 destroyed then keratinisation of some epidermal cells can occur resulting in scurs. Scurs usually
327 result from inadequate burning of the horn bud site in an effort to reduce the risk of thermal
328 injury to the skull and brain [22] or insufficient removal of the germinal tissue from the base
329 of the horn bud [15]. If the base of the horn bud, which can be hard to see, is wider than the
330 diameter of the cautery iron and not all tissue is destroyed, then scurs may also develop.
331 Interestingly, in many cattle breeds, there is a gene for scurs [30-32], as well as polled and
332 horned genes [33], meaning that scurs can grow naturally without disbudding. It is unknown
333 whether a gene for scurs exists in goats. If so, herds with high rates of scurs may be associated
334 with the genetic potential to grow scurs.

335 The best alternative to reduce pain and complications associated with cautery
336 disbudding, would be to breed polled goats to eliminate the need for this procedure. There are
337 hurdles to achieving this however, as the gene for polledness is linked with the intersex gene
338 in goats [34]. This means that there is a high probability that naturally polled goats will be
339 infertile. Until a polled line can be established, mitigating pain associated with cautery
340 disbudding or further alternatives that cause less pain or injury than cautery disbudding should
341 be considered.

342

343 **Conclusions**

344 Clove oil injection did not appear to be as successful at preventing scurs or horns as
345 cautery disbudding and therefore the methodology used in this study may not be useful for
346 disbudding goat kids (if having no scurs is a concern). Interestingly cautery disbudding by
347 removing the horn bud germinal tissue was more efficacious than leaving the horn buds intact,
348 suggesting that the former method may be more effective for preventing horns, scurs or scorns.
349 Future research should explore different clove oil formulations and administration methods or
350 other alternatives to cautery disbudding that may be efficacious and cause less pain.

351

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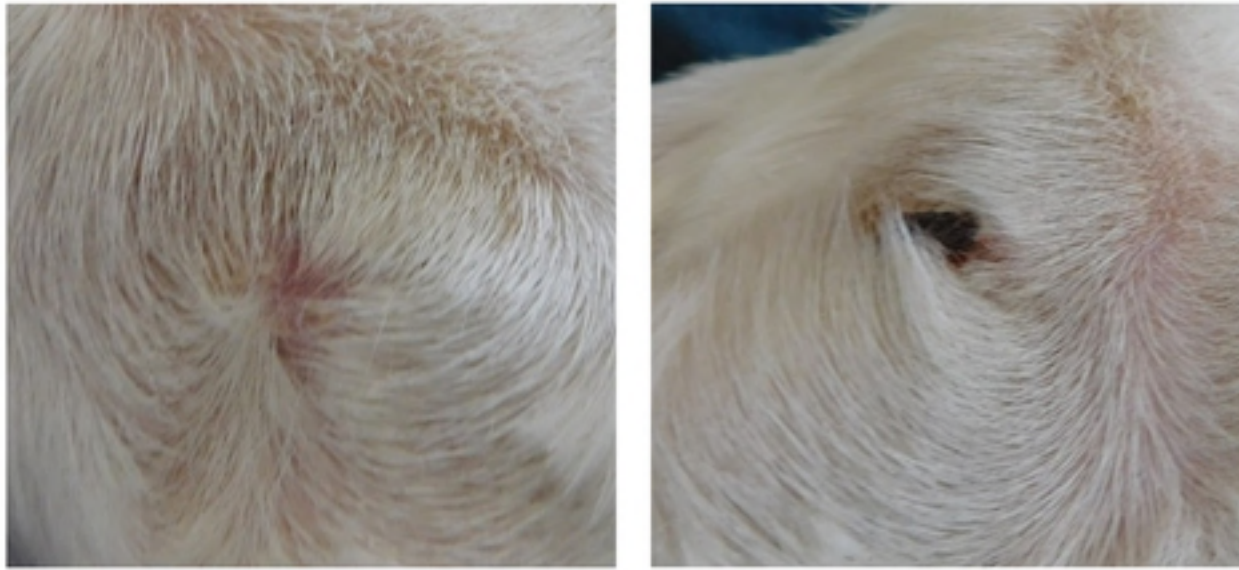
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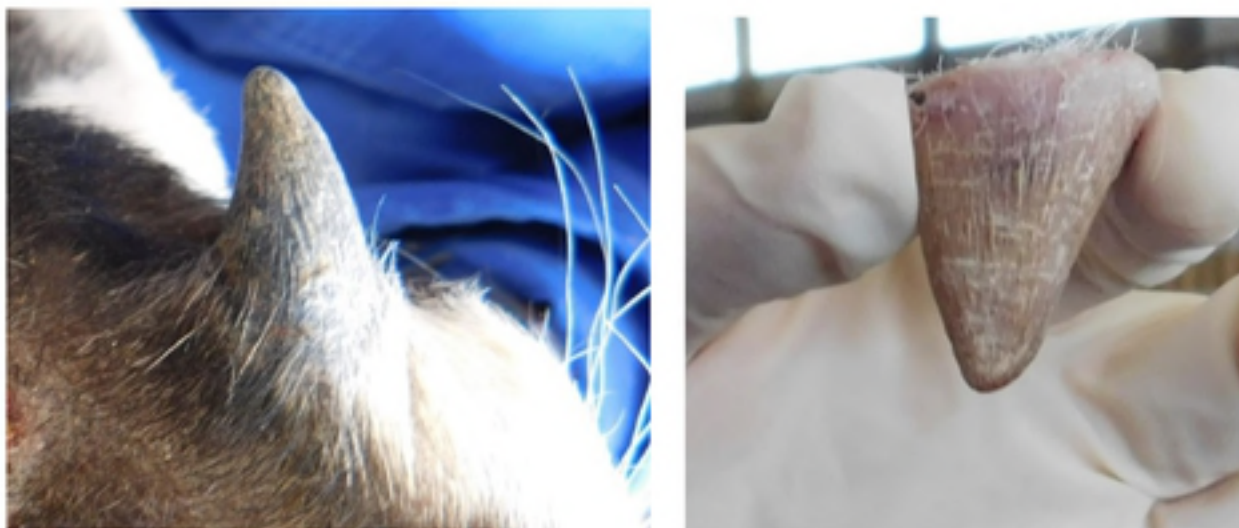
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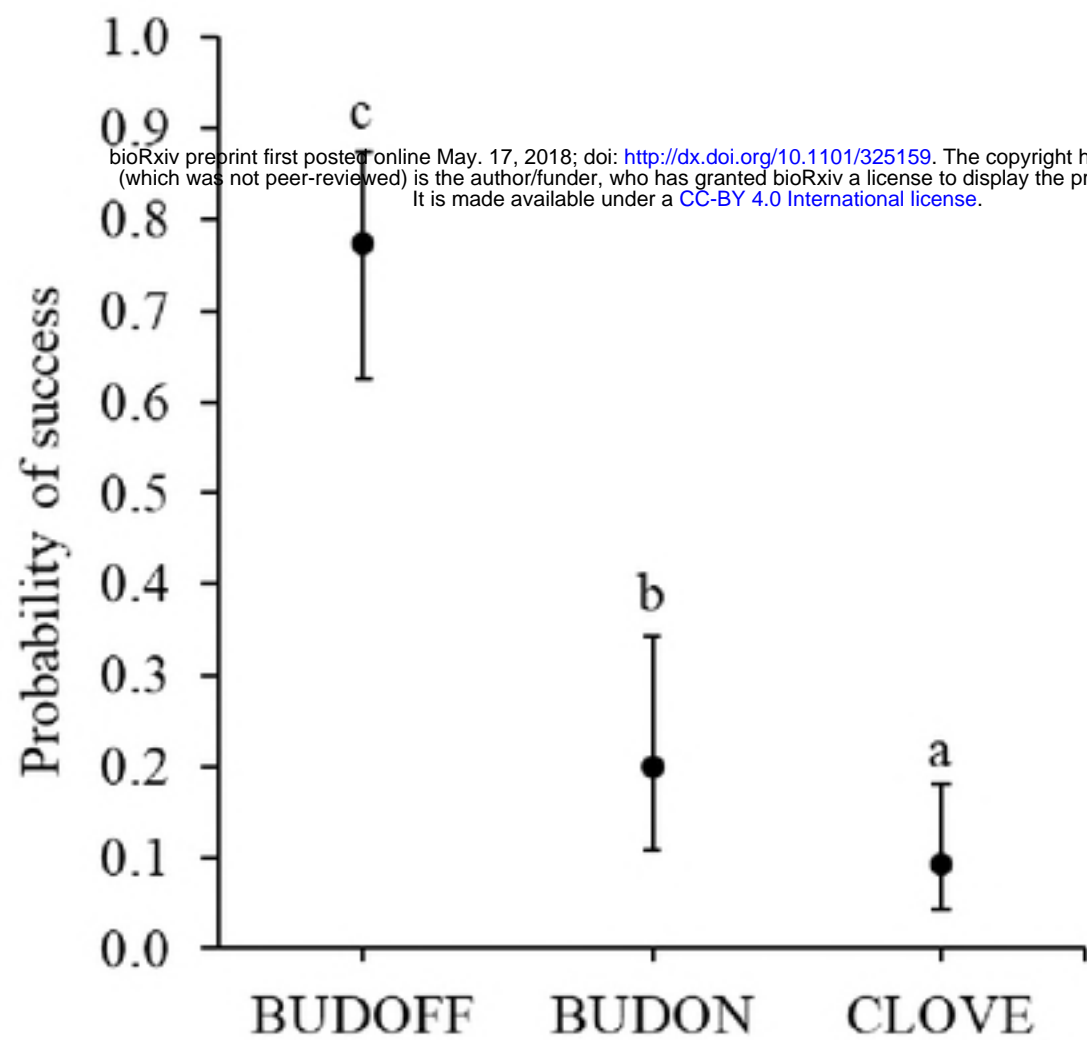
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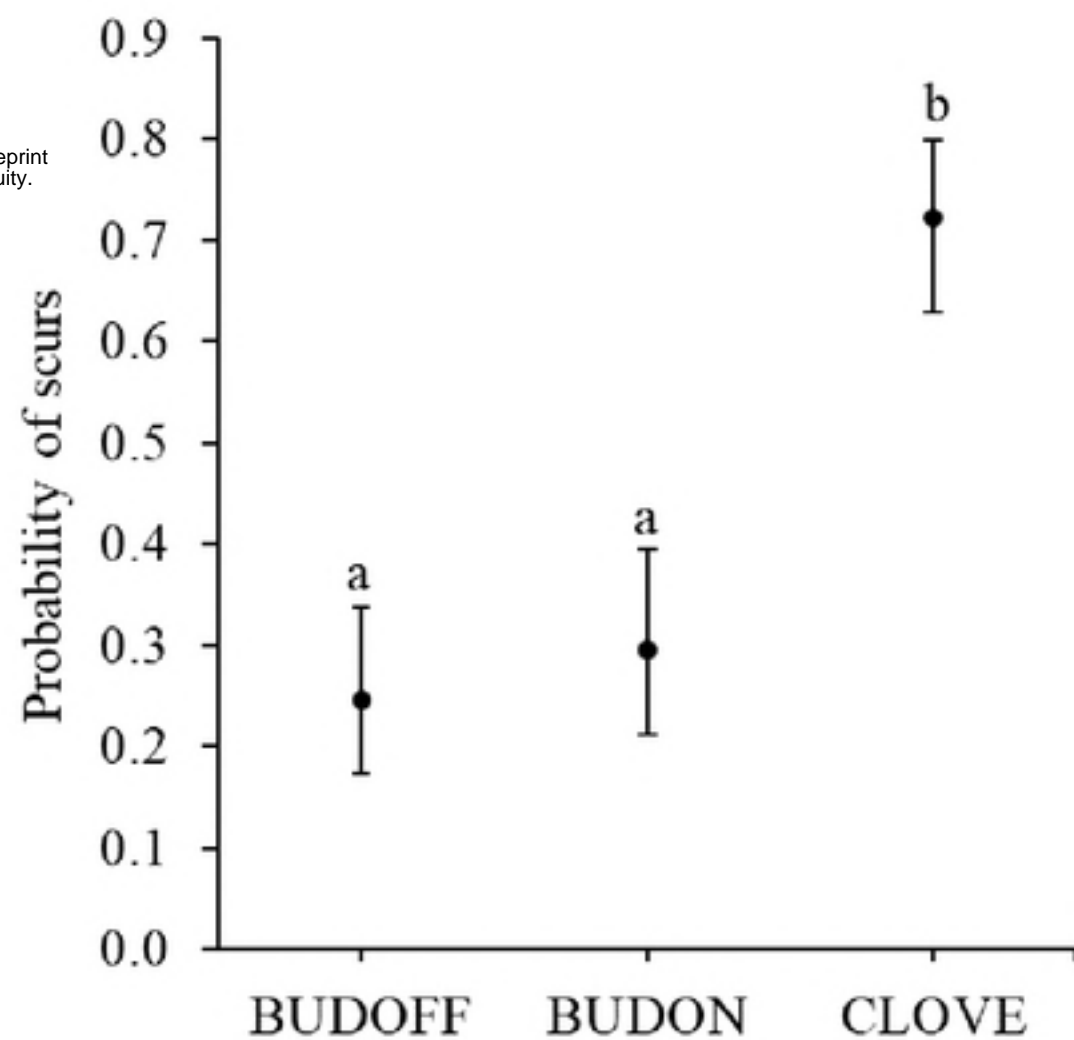
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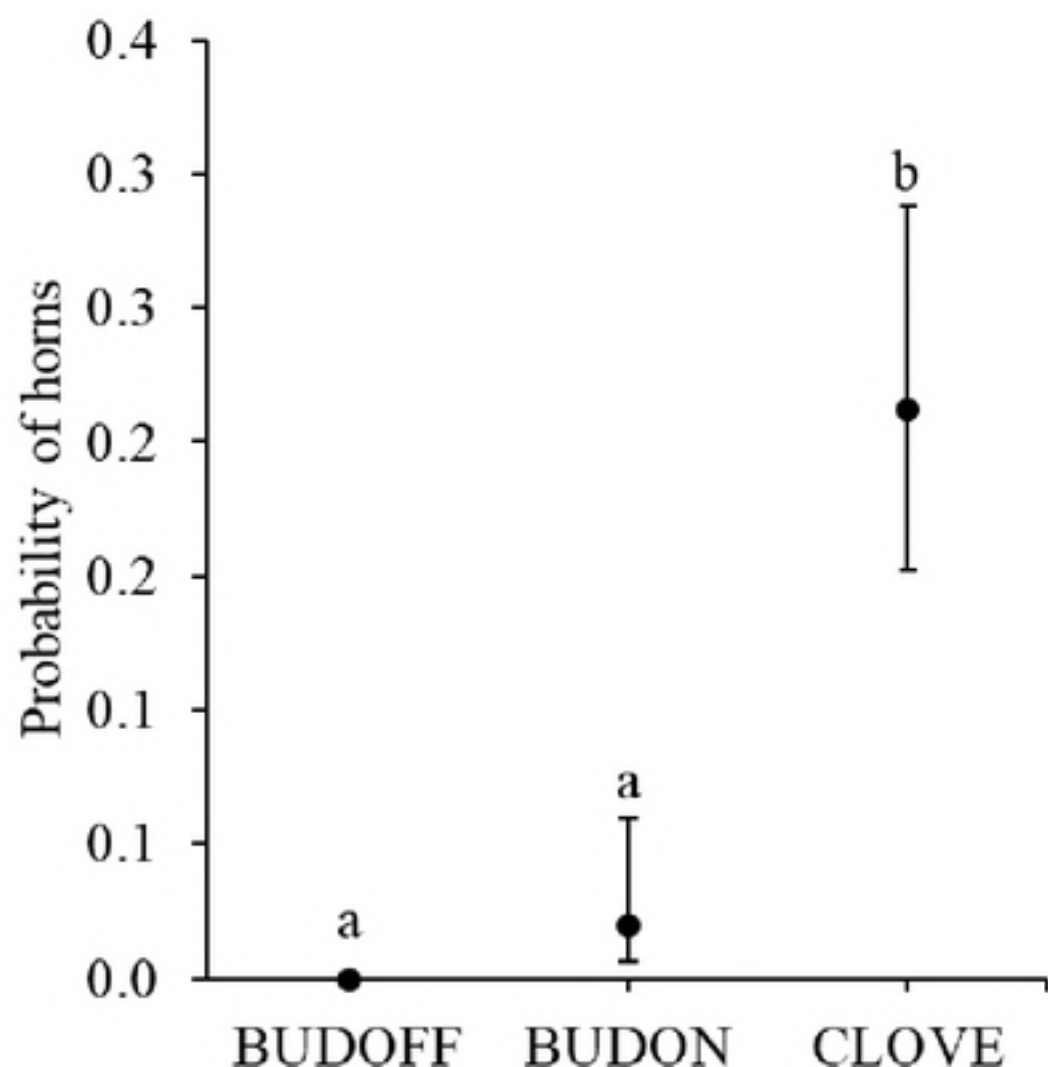
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