Is clove essential oil (Eugenia caryophyllata) able to chemically castrate newborn pigs?

O óleo essencial de cravo da índia (Eugenia caryophyllata) é capaz de castrar quimicamente suínos recém-nascidos?

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ABSTRACT

Chemical castration has been an alternative to reduce costs for producers and improve animal welfare. The clove (Eugenia caryophyllata) has several therapeutic potentials, among which there are reports of sterilizing potential. The aim of this study was to evaluate the potential sterilizing effect of clove (Eugenia caryophyllata) essential oil in pigs, in order to reduce costs to producers while at the same time following animal welfare guidelines. Twenty pigs were randomly selected and divided into four equal groups. Clove oil was administered at two concentrations in the animals of two groups (G1 = 0.07 mL/kg; G3 = 0.3 mL/kg)
and saline solution at different concentrations in the two remaining groups (G2 = 0.07 mL/kg; G4 = 0.3 mL/kg). At 60 days after induction of chemical castration, the animals underwent orchiectomy, a procedure in which the testicles were collected for histological processing. Histopathology revealed that none of the concentrations used was able to induce chemical sterilization of the animals, different from what was expected. It is concluded that the chemical castration technique is easy to perform, but the clove essential oil was not able to induce chemical sterilization in animals at the concentrations in which was used.

**Keywords:** Chemical castration; Essential oil; Pigs; Pig farming; Testicles.

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RESUMO

A proteína animal mais consumida no mundo é a carne suína. Para melhorar o sabor e a palatabilidade da carne, os produtores buscam melhorias de suas técnicas. Assim é necessária a ausência de hormônios esteroides que alteram a qualidade do produto. A castração química tem sido uma alternativa para reduzir custos aos produtores e melhorar o bem-estar animal. O cravo da índia (*Eugenia caryophyllata*) apresenta diversos potenciais terapêuticos, entre os quais há relatos de potencial esterilizante. O objetivo deste estudo foi avaliar o potencial efeito esterilizante do óleo de cravo da índia (*Eugenia caryophyllata*) em suínos, a fim de reduzir custos aos produtores e que sigam as diretrizes de bem-estar animal. Foram selecionados 20 suínos ao acaso e divididos em quatro grupos iguais. O óleo de cravo foi administrado em duas concentrações nos animais de dois grupos (G1 = 0,07mL/kg; G3 = 0,3mL/kg) e também solução salina em diferentes concentrações nos dois grupos restantes (G2 = 0,07mL/kg; G4 = 0,3mL/kg). Aos 60 dias após a indução da castração química os animais foram submetidos à orquiectomia, onde os testículos foram coletados para processamento histológico. A histopatologia revelou que nenhuma das concentrações utilizadas foi capaz de induzir a esterilização química dos animais, diferente do que era esperado. Conclui-se que a técnica de castração química é de fácil execução, porém o óleo de cravo não foi capaz de induzir a esterilização química em animais nas concentrações em que foi utilizado.

**Palavras-chave:** Óleo essencial; Porcos; Quimiocastração; Suinocultura; Testículos.

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INTRODUCTION

Pork has become the most consumed animal protein in the world (MERLINI et al., 2014; BERTOL, 2019). Its consumption had a significant increase, bringing great prospects for Brazil in the international market, making the country the fourth-largest producer and exporter of this product (ABPA, 2020).

Consumers are increasingly demanding about animal welfare, questioning breeding systems, the number of animals per area, slaughter conditions, and the quality of animal products obtained. Thus, improvements in animal management techniques are needed to increase the consumption of pork (BERTOL, 2019).

The castration of males performed at seven days of age, in addition to promoting improvements in meat quality, also aims to suspend the production of steroid hormones through
testicular inactivity. Thus, such substances would not provide unpleasant odors and flavors to the meat (FERREIRA et al., 2019). However, it is usually performed without anesthesia, not respecting the principles of animal welfare (SANTOS, 2016).

Immunocastration is an alternative technique that is widespread worldwide (FERNANDES et al., 2017). It consists of using the animal’s own immune system through the administration of a vaccine, which will prevent the action of the hypothalamic-pituitary-gonadal axis (HPG axis) through produced antibodies, interrupting testicular development and the production of steroid hormones (ABCS, 2014; TEIXEIRA; TOCCHET, 2014). However, it is still considered a high-cost procedure for producers (BRUNO et al., 2013).

A method considered promising that has been reported in the literature is chemical castration (JANA; SAMANTA, 2011), a non-surgical procedure (OLIVEIRA et al., 2012) based on the intratesticular administration of chemical agents, whose action is to promote definitive changes in the male reproductive system, causing azoospermia, in addition to inflammation and necrosis in these structures (KUTZLER; WOOD, 2006).

The administration of these chemical agents is done through a single dose for sterilization (KARAKUS et al., 2017; VARGAS et al., 2020), having as proof of effectiveness the confirmation of the absence of reproductive function in the animals tested, safety test to animals and the environment, in addition to irreversible results after administration (OLIVEIRA et al., 2012).

Although the technique is considered advantageous, SINGH et al. (2020) report the presence of intense pain during the procedure, recommending the use of local anesthetics to aid in the analgesia of animals.

Sclerosing agents are described in the literature for having a potential sterilizing effect, such as calcium chloride (KOGER, 1978), clove essential oil (ABSHENAS et al., 2013; ABU-AHMED, 2015), zinc gluconate associated with DMSO (Dimethyl sulfoxide) (LUCAS et al., 2016).

The use of plants has become common for therapeutic purposes (ESTEVÃO et al., 2013) due to the great dissemination of knowledge by the scientific community (PIRIZ et al., 2014). The clove (Eugenia caryophyllata) has been used in studies because it has antimicrobial, antioxidant, antifungal, antiviral, anti-inflammatory, cytotoxic, insect repellent, and anesthetic properties (CHAIEB et al., 2007).

Therefore, this study aimed to evaluate the sterilizing potential of clove essential oil (E. caryophyllata) to chemically castrate male pigs, in order to offer low-cost and easy-to-perform alternatives, in addition to following animal welfare guidelines.

**MATERIALS AND METHODS**

*Acquisition of clove essential oil (E. caryophyllata)*
The clove essential oil used in the experiment was purchased from the company Ferquima (Vargem Grande Paulista, São Paulo) by the Universidade Paranaense – UNIPAR.

Animals for the experiment

Twenty domestic pigs (*Sus scrofa domesticus*) (n = 5 per group), males, no defined breed, and seven days old, from the swine sector of the Universidade Paranaense (UNIPAR), were used. Before undergoing the proposed procedure, the animals were weighed and clinically examined (temperature, heart rate, mucosal color, respiratory rate, capillary filling time, and degree of hydration), only healthy animals were selected, with vital parameters within the reference standard.

All procedures were performed in accordance with the Ethics Committee for Research Involving Animal Experiments, Universidade Paranaense (CEPEEA/UNIPAR), Protocol 33359/2018. All regulations and recommendations from the National Council for the Control of Animal Experiments (CONCEA) were respected in order to provide the animals’ well-being.

Experimental groups and treatments

The animals were identified with earrings and separated according to the average weight. The dose used in the study was extrapolated from ABU-AHMED (2015).

G1 - Experimental group, receiving a dose/volume of 0.07 mL/kg of clove essential oil (*E. caryophyllata*) in each testicle.

G2 - Control group, receiving a volume of 0.07 mL/kg of saline solution in each testicle.

G3 - Experimental group, receiving a dose/volume of 0.3 mL/kg of essential oil of clove essential oil (*E. caryophyllata*) in each testicle.

G4 - Control group, receiving a volume of 0.3 mL/kg of saline solution in each testicle.

Chemical castration induction procedure

The chemical castration induction procedure was performed at seven days of age in the animals, in two distinct periods, being G1 and G2, G3 and G4, respectively. Before the procedure, Meloxicam was administered to all animals at a dose of 0.4 mg/kg intramuscularly (IM) for preemptive analgesia and then intratesticular infiltrative anesthesia was performed, using 2% lidocaine hydrochloride in the dose of 5 mg/kg in all animals (KEITA *et al*., 2010; BONASTRE *et al*., 2016). After 15 minutes of local anesthetic administration, intratesticular solutions were administered bilaterally, according to the respective treatments. The animals were clinically followed for seven days to check for any complications. After seven days, the animals were released from clinical care.
Orchiectomy Procedure

At 60 days after the induction of chemical castration, the animals were weighed and submitted to the bilateral orchiectomy procedure. For this, the animals were submitted to solid fasting for 12 hours for induction of anesthesia. Preoperatively, animals received Meloxicam at a dose of 0.4 mg/kg IM for preemptive analgesia and benzathine penicillin at a dose of 40,000 IU/kg IM. Subsequently, the animals received pre-anesthetic medication by administering azaperone at a dose of 4 mg/kg IM and intratesticular infiltrative anesthesia was performed with 2% lidocaine at a dose of 5 mg/kg. Subsequently, general anesthesia of the animals was performed using the association of xylazine in a 2.0% solution, tiletamine with zolazepam at a dose of 5 mg/kg, and atropine sulfate in a 1.0% solution, as described by PACHALY (2006).

The orchiectomy was performed through a scrotal incision, exposing the testicles. Then, the ligature of the spermatic cord was performed by transfixation, using a 3-0 Nylon monofilament suture. The incision remained open for spontaneous healing by secondary intention. Thus, a general inspection of the surgical site was performed, and the orchiectomy procedure was considered completed. The animals were followed for ten days, and dressings were performed daily by cleaning the wounds with saline solution.

Material collection and histological processing

After collecting the testicles in the surgical procedure, they were sectioned in their middle portion, preserving the testicular parenchyma, vascular plexus, and ducts. Afterward, they were fixed in 10% neutral buffered formalin (NBF) to avoid post-mortem degeneration and consequently cell autolysis. After fixation, the material went through a dehydration step by means of an increasing series of alcohols, diaphonization with xylene, and infiltration and embedding in paraffin. After blocking the structures, histological sections were performed in a rotary microtome with a thickness of 5 µm and stained with standard staining of Hematoxylin and Eosin (H&E). Histological slides were mounted using synthetic resin between slide and coverslip in order to preserve the material used.

Documentation and histopathological evaluation

The produced slides were photographed with the aid of a Motic® digital camera (Moticam 5.0 MP) coupled to an optical microscope (Nikon Eclipse E200®), with 4x, 10x, and 40x objectives, using the Motic Images Plus 3.0® software.

The structures of the tunica albuginea, testicular septa, and testicular lobes were analyzed, observing the structures of the seminiferous tubules, interstitial, and support cells. The mediastinum testis, rete testis, vas deferens, and epididymis were also evaluated (ABSHENAS et al., 2013; ABU-AHMED, 2015; OLIVEIRA et al., 2017; LEOCI et al., 2019).
RESULTS

At the end of the experiment, no complications or difficulties were observed in the execution of the technique used to induce chemical castration in pigs. In the macroscopic evaluation of the animals' testicles, no alterations that could show edema, hyperemia, signs of inflammation, and pain in the different treatments were observed. In the histopathological processing, it was observed that all evaluated structures remained intact 60 days after the application of treatments to the animals (Figures 1, 2, 3 and 4; Table 1).

Figure 1 - Microscopic photograph of swine testis at 60 days after intratesticular drug administration. (a) Group G1 treated with 0.07 mL of clove essential oil (b) Group G2 treated with 0.07 mL of saline solution. Convoluted epididymal ducts (red arrow) in the testicular lobes, separated by interstitial connective tissue septum (black arrow), structural changes not observed (10x objective).

Source: Authors, 2021.

Figure 2 - Microscopic photograph of swine testis at 60 days after intratesticular drug administration. (a) Group G3 treated with 0.3 ml clove essential oil (b) Group G4 treated with 0.3 mL of saline solution. Seminiferous tubules (black arrow) arranged in testicular lobes. Germ cells (red arrow) arranged in a centripetal shape in the seminiferous tubule, with no structural changes observed (10x objective).
Figure 3 - Microscopic photograph of swine testis at 60 days after intratesticular drug administration. (a) Group G1 treated with 0.07 mL of clove essential oil (b) Group G2 treated with 0.07 mL of saline solution. Convoluted epididymal ducts (black arrow), covered by preserved stereocilia (red arrow), with no structural changes observed (10x objective).

Figure 4 - Microscopic photograph of swine testis at 60 days after intratesticular drug administration. (a) Group G3 treated with 0.3 mL clove essential oil (b) Group G4 treated with 0.3 mL of saline solution. Convoluted epididymal ducts (black arrow), covered by preserved stereocilia (red arrow), structural changes not observed (10x objective).
Table 1 - Evaluation of testicular structures in swine submitted to intratesticular drug administration to identify morphological changes. Experimental groups (G1 = clove essential oil, dose 0.07 mL; G3 = clove essential oil, dose 0.3 mL) and controls (G2 = saline solution, dose 0.07 mL; G4 = saline solution, dose 0.3 mL).

<table>
<thead>
<tr>
<th>Histopathological Evaluation</th>
<th>Experimental Groups</th>
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<tbody>
<tr>
<td>Tunica albuginea (alteration), inflammatory response, congestion, and hemorrhage.</td>
<td>G1      0/5</td>
</tr>
<tr>
<td>Degeneration, vacuolization, decrease in germ cells, dissociation of germ cells in the seminiferous tubules.</td>
<td>G1      0/5</td>
</tr>
<tr>
<td>Formation of multinucleated giant cells, absence of elongated spermatids, and atrophic seminiferous tubules.</td>
<td>G1      0/5</td>
</tr>
<tr>
<td>Sertoli cells showing varying degrees of degeneration, apoptosis, and necrosis.</td>
<td>G1      0/5</td>
</tr>
<tr>
<td>Leydig cells showing varying degrees of lipid degeneration and necrosis.</td>
<td>G1      0/5</td>
</tr>
<tr>
<td>Epididymal duct with vacuolated tubular lining cells, hydropic degeneration of hair cells.</td>
<td>G1      0/5</td>
</tr>
<tr>
<td>Fibrosis of epididymal smooth muscle tissue, inflammatory infiltrates, and vessel congestion.</td>
<td>G1      0/5</td>
</tr>
<tr>
<td>Evaluation of sperm count in the tubular lumen.</td>
<td>G1      0/5</td>
</tr>
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</table>

G1 - Experimental Group 1; G2 - Control Group 2; G3 - Experimental Group 3; G4 - Control group 4.
0/5 - Zero out of five animals.
DISCUSSION

In the search for methods that respect animal welfare, the surgical castration technique has been replaced in Brazil, due to the fact that there is a normative instruction No. 113/2020, which establishes good animal management and welfare practices in commercial breeding swine farms. This standard mainly requires analgesia and anesthesia for surgical castration in pigs of any age (BRASIL, 2020). As a result, several studies seek new alternatives for producers using chemical castration as an option, a technique considered less invasive, more innovative and that reduces possible infections (LOPES; SILVA, 2014; SOTO, 2015), in addition to being considered an easy-to-perform technique in pigs (SILVA et al., 2019), as observed in the present study.

Although it is considered a technique that promotes severe pain in the animal (SINGH et al., 2020), when performed using an intratesticular local anesthetic, it is able to minimize such discomfort (ABSHENAS et al., 2013; ANDRADE NETO et al., 2014; ABU-AHMED, 2015). A protocol indicated for performing chemical castration is the use of meloxicam associated with local intratesticular anesthesia with lidocaine in pigs up to seven days of age (BONASTRE et al., 2016). This study was based on this protocol, and no signs of pain were observed in the animals after the administration of clove oil, which may be a result of the analgesic protocol used.

PEREIRA et al. (2018) carried out a study in cattle using 2% lidocaine in the spermatic cord of animals as local anesthesia and administered calcium chloride associated with DMSO for chemical sterilization. The authors did not observe signs of pain or sensitivity to manipulation of the site. PARANZINI et al. (2018) also used calcium chloride associated with DMSO to induce sterilization in cats. However, they do not report the presence of pain in animals, nor analgesia protocol before the administration of sterilizing agents.

ABSHENAS et al. (2013) used clove oil and saline solution in two different groups of dogs for chemical castration, under local intratesticular anesthesia with 1% lidocaine hydrochloride. However, the authors did not report complications after its administration in animals.

On the other hand, there are reports in the literature of pain signal observation in chemical castration protocols. ABU-AHMED (2015) used sedation with xylazine hydrochloride to perform an intratesticular injection of calcium chloride and clove oil in two different groups of dogs. The author observed discomfort during handling minutes after application, in both treatments, this discomfort persisting in the calcium chloride treatment after the first week.

SILVA et al. (2018) carried out a study in 12 dogs using calcium chloride associated with DMSO and saline solution in two distinct groups to induce chemical castration. For that, they performed anesthesia using intravenous propofol before administering the treatments, reporting pain on testicular palpation in an animal treated with calcium chloride associated with DMSO.

In this study, no macroscopic changes were observed in the testicles of the animals in the different treatments, possibly due to the protocol used before the administration of clove oil and
saline solution, as described by BONASTRE (2016). Other authors have also reported the absence of macroscopic changes in the testes of animals in their studies, using different intratesticular sclerosing agents (OLIVEIRA et al., 2012; ABU-AHMED, 2015).

After histopathological processing, in the microscopic evaluations of the testicles of animals in groups G1 and G3, which received clove oil at a dose/volume of 0.07 mL and 0.3 mL, respectively, no testicular morphological changes were evidenced that could cause sterilization of animals. Likewise, these alterations were also not observed in the control groups G2 and G4 (Figures 1, 2, 3 and 4 and Table 1).

In the literature, there are reports of testicular alterations that indicate the sterilizing potential of clove oil, as in the work of ABU-AHMED (2015), who, when using clove oil to chemically castrate dogs, observed different degrees of degeneration in the seminiferous tubules and interstitium, disruption of basal cells and necrotic cells in the tubular lumen, with loss of their normal morphology. Furthermore, when analyzing the epididymis, the author observed structural disarray, with thickening of the interstitium, in addition to few vacuolizations and thinning of the lining epithelium.

ABSHENAS et al. (2013) also used clove oil to induce chemical castration in dogs. The authors observed severe changes in the seminiferous tubules and interstitium, with the presence of severe diffuse tubular necrosis and different degrees of inflammatory response.

Other chemical agents were also used for the chemical sterilization of animals. PEREIRA et al. (2018) used calcium chloride associated with DMSO at different concentrations for chemical castration of cattle and observed coagulant necrosis of seminiferous tubules and interstitial cells, in addition to areas of liquefaction in most of the tissues observed. SILVA et al. (2018) also used calcium chloride associated with DMSO to induce chemical castration in dogs, observing suggested areas of seminiferous tubule degeneration, in addition to fibrosis associated with inflammatory infiltrates and the presence of intratubular calcification in most of the animals tested.

In all groups in this study, germ cells, spermatid, and spermatogonia were observed, reinforcing the absence of lesions in the seminiferous tubules, indicating that these animals were in the process of spermatogenesis, and that, conceptually, structural changes in tissues and germ cells mean reduced fertility in males (GARCIA, 2017). Thus, it is possible to reinforce the conclusion that the animals did not show positive results regarding the chemical castration process. In other works, authors who report the castration process in their studies describe the absence of germ cells, as well as the absence of their regeneration (ABSHENAS et al., 2013; ABU-AHMED, 2015; PEREIRA et al., 2018; SILVA et al., 2018).

In general, the events that trigger puberty in pigs start at five months of age, with sexual maturity around 10 months (HAFEZ & HAFEZ, 2004). This factor may explain the ineffectiveness of castration with clove oil in the model adopted in this experiment, as the animals
were submitted to intratesticular injection at seven days of life, simulating the moment of mechanical castration performed mainly by small producers. Therefore, the animals were in structural development, that is, too immature to undergo a chemical castration process.

**CONCLUSIONS**

The technique for the induction of chemical sterilization using the sclerosing agent of the essential oil of cloves (E. caryophyllata) proved to be easy to carry out with the possibility of generating lower costs for producers. However, the agent was not able to induce chemical sterilization in pigs, as demonstrated in macroscopic and microscopic findings. It is possible to infer that the immaturity of animals may be related to the lack of effectiveness of the sclerosing agent. Further studies are needed to clarify the sclerosing agent in question, especially in animals that are entering the stage of sexual maturity.

**REFERENCES**


