

**FEDERAL UNIVERSITY OF PAMPA
POSTGRADUATE PROGRAM IN ANIMAL SCIENCE**

**EFFECT OF PERCUTANEOUS ULTRASOUND-GUIDED INTRA-
OVARIAN INJECTION OF CALCIUM CHLORIDE IN RATS**

Masters Dissertation

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Urugaiana, Rio Grande do Sul, Brazil

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Dissertation presented to Postgraduate program in
Animal Science (*Stricto Sensu*) of
Federal University of Pampa, as partial
requirement to obtain the title of Master's in
Animal Science.

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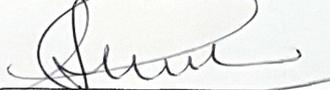
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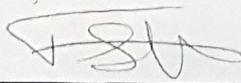
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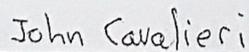
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Motivation comes from working on things we care about. It also comes from working with people we care about.

- Sheryl Sandberg

ABSTRACT

The overpopulation of free-roaming dogs and cats is a worldwide problem. These animals are subject to different types of situation, as well as providing greater circulation of zoonoses, which becomes a risk to public health. The main method of population control is the sterilization of animals, with surgical procedures (ovariohysterectomy and orchiectomy) being the most commonly performed. Thus, alternative methods of castration are being developed in order to reduce the costs and possible complications of the procedure, as well as to enable the large-scale use of alternative procedures in a manner that is safe and humane for animals. In this context, calcium chloride has already been tested for sterilization in male dogs and cats through its intra-testicular application, obtaining excellent results. The present study aimed to evaluate the viability and outcomes of the intraovarian application of calcium chloride in rats aiming at the induction of ovarian atrophy and, assessing its future potential for undertaking studies in bitches and queens. After standardizing the volume to be injected, 30 animals were divided into three groups: physiological saline solution (GC); 10% calcium chloride diluted with 95% ethanol (G1); 20% calcium chloride diluted with 95% ethanol (G2). The groups were subdivided into two evaluation times, 15 days (n = 5 of each group) and 30 days (n = 5 of each group). The estrous cycle of the animals was monitored daily until euthanasia using the cytology technique by vaginal lavage. To determine the ovarian measurements, ultrasound evaluations were performed in three moments: prior to the injection; immediately after application; and immediately after euthanasia. In addition, macroscopic and microscopic evaluation of the ovary was performed in order to identify possible lesions induced by the action of calcium chloride. In the present study, the use of calcium chloride diluted in 95% ethanol demonstrated it to be a potential agent to induce ovarian damage in rats without clinical side effects. In addition, the ultrasound guided intra-ovarian percutaneous injection technique presented good results for standardization and application in rats.

Keywords: chemical castration; minimally invasive technique; ovary; ultrasonography; estrous cycle; vaginal cytology.

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1 INTRODUCTION

The overpopulation of stray animals is considered a worldwide problem, which may cause transmission of zoonoses, accidents and uncontrolled species reproduction (JACKMAN; ROWAN, 2007). Sterilization of animals is one of the main methods of population control, being the surgical procedures (ovariohysterectomy and orchietomy), the most applied techniques in small animals (CATHEY; MEMON, 2010). However, alternative castration methods are being researched in order to decrease the costs of surgical procedures, post-operative complications and enable its use safely on a large scale (JANA; SAMANTA, 2006).

The use of chemosterilizing substances such as zinc gluconate, calcium chloride (CaCl_2) and hypertonic saline solution have been reported (MOGHEISEH et al., 2017; NETO et al., 2014; PARANZINI et al., 2018). When compared to the surgical method, they have lower cost and demand less postoperative care (ABD-ALLAH et al., 2013; CAVALIERI, 2017; ROY et al., 2017). According to Cavalieri (2017), the most widely used chemosterilizers and the most developed experimental studies regarding their efficacy and side effects in domestic animals were CaCl_2 and zinc gluconate, the latter with greater disadvantages.

There is also a report of the use of CaCl_2 evaluating the effectiveness of the compound by intraovarian route in heifers (CAVALIERI; HAYES, 2017). However, it has not yet been established whether the use of the CaCl_2 in dogs, by intraovarian route, would also lead to tissue damage and sterility. Considering the unrestricted increase in wandering animals worldwide, it is necessary to develop new sterilization techniques not only for males, but also for females.

Therefore, the present work aims to evaluate the applicability of calcium chloride injection, by ultrasound guided technique, in different concentrations by intraovarian route in rats. With the results found in this study, future researches in bitches and queens may be developed.

4 SCIENTIFIC REPORTS

4.1 Chapter 1

IS THE RAT OVARY ACCESSIBLE BY ULTRASOUND GUIDED INJECTION FOR CHEMICAL STERILIZATION STUDIES?

ABSTRACT

The aim of this study was to describe a technique for ultrasound guided, percutaneous, intra-ovarian injection in Wistar rats. In some species, such as humans, equines and bovines, intraovarian administration of chemicals or needle aspiration of the ovary is undertaken. In rodents, which are widely used in scientific research, no technique has been described that allows intra-ovarian injection, without surgical exposure of the organ. In this study, 0.9% sodium chloride was administered through ultrasound guided, percutaneous injection into rats' ovaries. The ovaries were measured by ultrasound imaging before and immediately after injection and a significant increase in ovarian diameter was recorded. In addition, no clinical abnormalities were detected within 15 days of administration. The findings in this study indicate that intraovarian administration of saline could be conducted successfully without invasive surgery and was able to modify ovarian size. These results demonstrate that the technique of ultrasound-guided intra-ovarian percutaneous injection in rats is possible, which should enable standardization of the technique and application in rats.

KEYWORDS: Minimally invasive technique; ultrasonography; intraovarian; measurements.

4.2 Chapter 2

EVALUATION OF THE EFFECT OF INTRAOVARIAN ADMINISTRATION OF CALCIUM CHLORIDE DILUTED IN 95% ETHANOL ON RAT OVARIES

ABSTRACT

Different fertility control methods are researched as a measure for population control of stray animals worldwide. Chemical castration using calcium chloride has been widely studied over the years in males, but there are few studies related to its use in females. The objective of this work was to evaluate the effects of two concentrations of calcium chloride when administered by ultrasound-guided, percutaneous, intraovarian injection in rats. Thirty Wistar rats were randomly divided into three treatment groups (n = 10/group), which consisted of the intraovarian injection of: physiological saline solution (GC); 10% calcium chloride diluted with 95% ethanol (G1); 20% calcium chloride diluted with 95% ethanol (G2). The animals were subdivided into two evaluation times, 15 days (n = 5 of each group) and 30 days (n = 5 of each group). Ovarian ultrasound measurement revealed statistical difference ($p < 0,05$) between ovarian sizes before and immediately after the injection. However, no statistical difference was observed between groups at the measurements after euthanasia. At gross inspection, adhesions and ovarian cysts were noticed in G1 and G2. Histopathology analysis revealed presence of calcium deposition, changes in ovarian architecture, presence of giant cells and vessel congestion in G1 and G2. This is the first study that reports the use of calcium chloride diluted in 95% ethanol in rats' ovaries, which has been shown to be a potential agent for inducing ovarian injury.

KEYWORDS: ultrasonography; ovary; chemical sterilization; percutaneous technique; castration.

5 CONCLUSIONS

The present study demonstrates the possibility of performing ultrasound-guided percutaneous, intraovarian injection in rats. Thereafter, the application of chemosterilizers in a minimally invasive approach was thought to be possible in rats. The application of calcium chloride diluted in 95% ethanol using this method of administration was demonstrated to be capable of inducing ovarian lesions in the evaluated time. However, it is suggested that more research should undertake in the future to assess its utility and efficacy in clinical applications.

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