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# Ovarian morphometry applied to the use of chemosterilant in bitches and queens

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ABSTRACT

Chemical sterilization as an alternative method to surgical castration, have been studied. However, considering females, it is necessary to know adequate volumes of chemosterilizer to be injected into the ovary and biological factors that influence ovarian volumetry. This study aimed to determine the volume of 20% calcium chloride solution diluted in 95% ethanol to inject into the ovary of bitches and queens. Furthermore, correlations between ovarian measures (length [L], width [W], height [H], and volume) and body mass, age, and the volume of injected chemosterilizer were analyzed. For this, 64 ovaries, collected after elective ovariohysterectomy, received an injection of the solution. Ovarian measures were performed pre-injection and post-injection, and volume was calculated using Lambert's empirical formula, the formula for an ellipsoid, and the formula for spherical prolate. The mean ± standard error of the chemosterilant volume injected in the ovary was 0.09 ± 0.02 mL for bitches and 0.10 ± 0.02 mL for queens; ovaries' width obtained a strong correlation with the injected volume; age showed a correlation to chemosterilant volume for both species and ovarian volume of queens; and body mass showed a correlation to ovarian measurements, ovarian volume e injected volume for bitches. Lambert's empirical formula and ellipsoid formula were more reliable for this assessment and respectively demonstrate an increase of 33% and 24% in ovarian volume in bitches post-injection; and 27% and 22% in queens post-injection. The use of chemosterilant beyond the possible limits of the organ may leakage in the abdominal cavity of females.

## 1. Introduçtion

The overpopulation of dogs and cats worldwide is a serious problem for public health (Lima; Luna, 2012). The most effective way to reduce the number of stray dogs and cats is massive sterilization, so alternative non-surgical methods have been developed to sterilize pets, such as immunocontraception and chemical castration (ACC&D, 2013). Among chemicals, 20% calcium chloride diluted in 95% ethanol has been used in dogs by intratesticular injection to induce sterilization (Leoci et al., 2019).

In males, the optimal volume of chemosterilizers to be injected in testis is based on its dimensions (Leoci et al., 2014). Only one study was found referring chemical castration in bitches with zinc gluconate (Mogheiseh et al., 2017), which based the chemosterilant volume considering the pattern of measurements previously determined for canine testicular injection (Oliveira et al., 2012). However, no scientific data was found that indicates this transposition is valid. Considering queens, there are not yet reports of the use of ovarian chemosterilants.

We hypothesized that there are correlations between age and body mass with ovarian volume and that ovarian measurements are associated to the chemosterilant volume needed for total organ fulfillment in dogs and cats. Then, this study aims to: (a) Define the appropriate volume of chemosterilant for repletion of the ovaries; (b) Check which variables correlate with injection volume (c) Check if body mass and age are factors that influence ovarian volume; (d) Compare and correlate three different formulas for calculating ovarian volume, and (e) Quantify the percentage of ovarian expansion after injection of chemosterilant.

## 2. Material and Methods

### 2.1. Ethical aspects

This study was approved (nº 025/2019) and followed the guidelines of the Ethics Committee on the Use of Animals (CEUA), and Normative Resolution No. 30/2016 of the National Board of Control of Animal Experimentation (CONCEA).

### 2.2. Animals and design experiment

The study was carried out with 16 bitches and 16 queens of different breeds and ages, considered clinically healthy and without signs of estrus, that underwent elective ovariohysterectomy as a sterilization method. Immediately after the ovariohysterectomy, ovaries, fallopian tubes, and uterine horns were maintained in saline solution (0.9% saline solution) and measurements were performed by the same technician. Ovarian length, width, and thickness were measured with a digital electronic caliper (accuracy: ± 0.2 mm; margin of error: 0.01 mm) and compiled (pre-injection measurements). The exclusion criteria used were morphological changes in the ovaries caused by the presence of cystic structures. Weight, breed, and age were also compiled.

Following the measurements, the bursa of the canine's ovaries was exposed and intraovarian injection was performed with a needle (26G) attached to a 1 mL syringe calibrated at 0.02 mL intervals. The injected solution was previously prepared and consisted of 20% calcium chloride diluted in 95% ethanol plus a drop of methylene blue dye for each 1 mL of solution, to evidence the total fill of the ovary and leakage.

The needle was inserted through the caudal pole of the ovary in a horizontal position, towards the cranial pole without any cranial perforation while the organ was digitally restrained. The solution was injected as the needle was gradually removed from the organ until fulfilling and stain were noticed (Figure 1A). The injection was interrupted, and the needle was removed from the organ, so the injected volume was recorded. Then, the measurements (length, width, and thickness) were undertaken and compiled again (post-injection).

The three variables measured were used to estimate the ovarian volume pre- and post-injections. Ovarian volumes (OV) were calculated using three different formulas, as follows: (OV1): Lambert's empirical formula = L (length) × W (width) × H (height) × 0.71 (LWH0.71); (OV2): Ellipsoid formula = L × W × H × 0.52 (LWH0.52); and (OV3): Spherical prolate formula = L × W2 × 0.52 (LW20.52) (Pilatz et al., 2012).

### 2.3. Statistical Analysis

Results of body mass, age, injected volume, and ovarian measurements (length, width, thickness, and volume) pre-injection and post-injection obtained were analyzed using descriptive statistics (mean ± standard error, minimum and maximum). Correlations of ovarian measurements, body mass, age, ovarian measurements, ovarian volume, and volumes of chemosterilant injected were investigated. Normality tests (Shapiro-Wilk and Kolmogorov-Smirnov) were performed. When data were normally distributed, Student's T-test was used to compare means and the Pearson test was used for correlations. When data were not normally distributed, the Wilcoxon test was used to compare means and the Spearman test was used for correlations.

The analyzes were performed using IBM SPSS® Statistics software (version 20.0). Significant differences were considered when $p < 0.05$ and the correlation values presented by the correlation coefficient "r" (-1 to 1). It was considered a weak correlation when the coefficient was (0.10-0.39), a moderate correlation (0.40-0.69), a strong correlation (0.70-0.89), and a very strong correlation (0.90-1.00). Linear graphs and percentages of variations of measures and volumes were made using Microsoft Excel Software (version 2013).

## 3. Results

From the 32 ovaries from each species, were included 31 canine and 23 feline ovaries in the study. The remaining ovaries were excluded because cystic structures were identified. The mean body mass of the bitches was 11.96 ± 6.64 kg (minimum of 3.6 and maximum of 32.0 kg) and mean age 28.25 ± 23.52 months (minimum of 6 and maximum of 96 months). Different breeds were included: crossbred (n = 10), Lhasa (n = 1), Border Collie (n = 2), Australian Cattle Dog (n = 2) and Golden Retriever (n = 1).

In the feline group, the mean body mass was 2.67 ± 0.48 kg (minimum of 2 and maximum of 3.6 kg) and mean age 13.82 ± 5.33 months (minimum of 7 and maximum of 24 months). Most of the cats were crossbred (n=15) and one was Persian. The presence of unilateral cystic structures was found mainly in cats (Figure 1B).

A minimal leakage of the solution was observed. This leakage occurred when the ovary was already filled to its maximum capacity, so fluid overflowed out of the organ. It was also observed staining of blood vessels towards the uterine horns, broad ligaments, and round ligaments (Figure 1C). In the ovaries of queens, there was a greater resistance of solutions overflow than in canine ovaries.

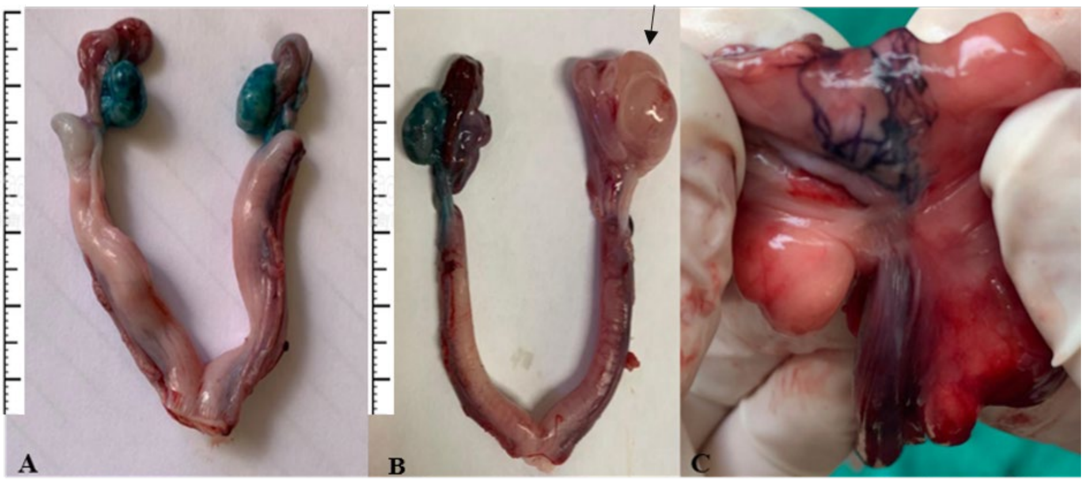


***Figure 1*** *– Photomacrography of the surgical specimen, showing left and right canine ovaries completely stained after chemosterilant injection, fallopian tubes, and uterine horns (A). Feline left ovary completely stained, the right ovary showing an ovarian cyst (black arrow) (B) and ovarian blood vessels stained (C).*

The mean of the injected volume was 0.09 ± 0.02 mL (minimum of 0.06 and maximum of 0.18 mL) in the bitches, while in queens, the mean was 0.10 ± 0.02 mL (minimum of 0.06 and maximum of 0.15 mL).

The mean of length, width, thickness (pre-injection and post-injection) are presented in Table 1 and were compared ($P < 0.05$) in a 95% confidence interval. In the canine group, the width and thickness showed a significant increase post-injection (11% and 21%, respectively); while in the feline group thickness increased (37%) and length showed a decrease post-injection (-5%). Considering ovarian volumes, results showed an increase in organ volume post-injection of bitches of 33% (OV1), 24% (OV2), and 18% (OV3). In the felines, ovarian volumes calculated with the formulas OV1 and OV2 also showed a significant increase post-injection, of 27% and 22%, respectively, and a decrease of 8% considering the OV3 formula (Table 1).

| Species | Ovarian Measurements | Measurement Pre injection (mm/mL) | Measurement Post injection (mm/mL) | Variation (%) | P - Value |
|---|---|---|---|---|---|
| Canine | Length | 11.86 ± 2.72 | 11.54 ± 2.61 | -3% | 0.245 |
| | Width | 6.35 ± 1.86 | 7.02 ± 1.69 | 11% | 0.001* |
| | Thickness | 4.70 ± 1.49 | 5.71 ± 1.34 | 21% | 0.001* |
| | OV1 | 0.27 ± 0.11 | 0.36 ± 0.14 | 33% | 0.001* |
| | OV2 | 0.21 ± 0.09 | 0.26 ± 0.11 | 24% | 0.001* |
| | OV3 | 0.28 ± 0.12 | 0.33 ± 0.11 | 18% | 0.002* |
| Feline | Length | 9.36 ± 1.38 | 8.91 ± 1.52 | -5% | 0.043* |
| | Width | 4.93 ± 0.77 | 4.86 ± 0.88 | -1% | 0.637 |
| | Thickness | 3.50 ± 0.79 | 4.81 ± 0.86 | 37% | 0.001* |
| | OV1 | 0.20 ± 0.04 | 0.24 ± 0.07 | 27% | 0.001* |
| | OV2 | 0.15 ± 0.03 | 0.18 ± 0.04 | 22% | 0.001* |
| | OV3 | 0.22 ± 0.04 | 0.23 ± 0.05 | -8% | 0.386 |

*Significant level at ($P < 0.05$)

***Table 1*** *– Description of ovarian measurements (length, width and thickness in mm) and ovarian volumes (OV1, OV2, OV3 in mL), calculated by three different formulas, pre-injection and post-chemosterilant injection (mean ± SD) in bitches and queens.*

In the canine group, body mass and length's ovaries presented a strong positive correlation. Width was the only canine ovarie's measure that strongly correlated to the three volume formulas. Injected volume had a positive correlation to canine ovarian volumes calculated by the three formulas. In the feline group, length's ovaries had a strong correlation with ovarian volumes but injected volume correlated only with OV3 (Table 2).

| Species | Measure | Measure | | | | | |
|---|---|---|---|---|---|---|---|
| | | Injected Volume | Length | Width | Thickness | Body mass | Age |
| Canine | Length | 0.189 | - | - | - | - | - |
| | Width | 0.453* | 0.448* | - | - | - | - |
| | Thickness | 0.440* | 0.227 | 0.724* | - | - | - |
| | Body mass | 0.207 | 0.787* | 0.445* | 0.240 | - | - |
| | Age | 0.633* | 0.096 | 0.038 | 0.160 | -0.43 | - |
| | OV1 | 0.435* | 0.621* | 0.873* | 0.840* | 0.595* | 0.112 |
| | OV2 | 0.433* | 0.624* | 0.861* | 0.845* | 0.592* | 0.110 |
| | OV3 | 0.413* | 0.672* | 0.930* | 0.633* | 0.614* | -0.140 |
| Feline | Length | 0.462* | - | - | - | - | - |
| | Width | 0.468* | 0.593* | - | - | - | - |
| | Thickness | -0.043 | 0.486* | 0.156 | - | - | - |
| | Body mass | -0.630 | -0.169 | -0.077 | 0.253 | - | - |
| | Age | -0.562* | -0.462* | -0.462* | 0.248 | 0.222 | - |
| | OV1 | 0.244 | 0.829* | 0.635* | 0.803* | 0.107 | -0.111 |
| | OV2 | 0.252 | 0.848* | 0.615* | 0.793* | 0.130 | -0.074 |
| | OV3 | 0.510* | 0.773* | 0.954* | 0.256 | -0.101 | 0.431* |

*Significant difference ($P < 0.05$)

***Table 2*** *– Correlations coefficient between ovarian pre-injection measurements, ovarian pre-injection volumes calculated by OV1, OV2 and OV3 formulas, the volume of injected chemosterilant, body mass, and age for canine and feline.*

## 4. Discussion

It was reported averages of ovarian widths varying from 12.0 ± 0.6 mm to 15.7 ± 0.3 mm in canines (Cunha et al., 2019). The same authors observed that ovaries of feline females measured about 11 ± 2 mm in width (Cunha et al., 2019), values that surpass the ones in this study. The differences found in ovaries' parameters could be explained by variations in measuring equipment, examiner, and individual biological factors.

Another report of ovarian morphometric evaluation in bitches, where the mean length ± standard error found was 13.41 ± 0.50 mm and width of 7.81 ± 0.31 (Binsfeld et al., 2014). In felines, it was observed that the mean ± standard deviation of the ovaries' length was 10.5 ± 1.6 mm (Gatel et al., 2020). The close data to those in the present study can be explained since cats have a more standardized size.

In a study using an intraovarian injection of zinc gluconate in bitches, the volume of injection was determined by ultrasound measurement of ovarian diameters. It was reported that the injection reduced the size of the ovary by the end of the study in 30 days (13.09 to 9.45 mm) (Mogheiseh et al., 2017). The authors used as reference the volume of injection of zinc gluconate established per testis width in dogs. In that study, authors supposed that as the volume chemosterilant used in the testis can be estimated by the organ's width (Oliveira et al., 2012), the same parameter could be used for females, but it was not found data proving that this transposition of values is valid. It was considered about 0.1-0.2 mL of zinc gluconate for injection in each ovary (Mogheiseh et al., 2017), values that surpass the ones reported here.

Although it was not described leakage of the chemosterilant (Mogheiseh et al., 2017), our study observed minimal leakage of the solution was in most ovaries of both species. It was also reported that fibrous tissue and the capsule around the ovary caused resistance to injection sometimes and could contribute to the escape of fluid from the ovary in cows (Cavalieri; Hayes, 2017). An experiment of injection in canine ovaries, leakage was reported, but also the bursa helped to contain the liquid (Parsemus foundation, 2010). In the present study, the bursa was removed from the canine ovaries to allow a clearer view and more accurate measurements of the organs.

Moreover, it was possible to notice that the solution stained the ovarian blood vessels towards the uterine horns, broad ligaments, and round ligaments, which were also reported previously (Parsemus foundation, 2010). This could be explained by the existence of anastomoses between uterine and ovarian arteries (Khafi et al., 2018). Therefore, the solution cannot be harmful to other tissues in contact, as an escape through the blood vessel system or into the abdominal cavity should be expected after the injection.

A study using the ellipsoid formula to calculate ovarian volume with ultrasound measurements of healthy girls' ovaries found that a gradual increase in all ovarian measurements happened with age, and ovarian parameters were significantly correlated to age, height, and weight, and stages of puberty (Razzaghy-azar et al., 2011). In this study, canine body mass had a positive correlation to ovarian measurements and volume, while age also was correlated to ovarian volume in felines, which stands out the importance of including these variables in ovaries experiments. In women, there is no consensus on the most suitable equation for estimating ovarian volume (Ali; Elsadawy; Khater, 2016).

Ovaries are three-dimensional ovoid structures that can change position, size, and volume through life (Hassen, 2013). The three-dimensional shape explains why a decrease in ovarian volume was observed after chemosterilant injection using the Spherical prolate formula (OV3) in the feline group. In OV3, the variable thickness is not present, influencing those results and showing a contradictory decrease in organ volume, since this did not happen biologically. For this reason, Lambert's empirical formula and the formula of an ellipsoid should be more reliable to estimate and compare variations in ovarian volumes, rather than the formula for a spherical prolate.

The information of how much ovarian volume expands post-injection (Lambert's empirical formula: 33% for bitches and 27% for queens; or the formula of an ellipsoid: 24% for bitches and 22% for queens), is an indirect parameter to assess whether the injection caused total organ repletion in future experiments. In this study, it was not measured the real ovarian volume (by liquid dislocation, for instance) since it was necessary to maintain the organ connected to its tissues to observe leakage. However, the formulas presented can be used in ultrasound measurements of ovaries in live animals, as it is done in bitches (Barbosa et al., 2013), humans (Razzaghy-azar, 2011), yellow-breasted capuchin (*Sapajus xanthosternos*), and robust tufted capuchin (*Sapajus robustus*) (Pissinatti et al., 2019).

Other factors beyond organ measurements affect the volume of chemosterilant that is supported by ovaries. So, it is necessary to include biological variables, such as age and weight, in future studies of ovary volumetry and chemosterilants. Volume determination should include a great number of animals, divided per group considering: age range, weight range, estrous cycle, body score, breed, and real organ volume. A low number of research studies concerning chemical injection in ovaries have been published and further evaluation using a larger population, evaluation of fluid leakage, long time follow-up, adjustments of dose, site of administration, and the components of treatment are important.

## 5. Conclusion

In this study, it was concluded that (a) an average of 0.09 mL for bitches and 0.1 mL for queens of the volume of chemosterilant was injected intraovarian; (b) width presented a strong correlation with the injected volume (c) Age showed a significant correlation to chemosterilant volume for cats and dogs and to ovarian volume of queens; body mass showed a significant correlation to ovarian measurements, ovarian volume and injected volume for bitches; (d) Lambert's empirical formula ($L \times W \times H \times 0.71$) or the formula of an ellipsoid ($L \times W \times H \times 0.52$) were more reliable for organ volume calculation and (e) According to Lambert's empirical and formula of an ellipsoid, an increase of 33% and 24% in ovarian volume was seen in bitches; and in queens, the organ volume increased 27% and 22%, respectively. The use of a volume of chemosterilant beyond the possible limits of the organ may leakage in the abdominal cavity and its clinical consequences are still unknown, but it is important to highlight that it can be harmful to live animals and should be better investigated.

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**Ethics approval:** This study was approved (nº 025/2019) and followed the guidelines of the Ethics Committee on the Use of Animals (CEUA), and Normative Resolution No. 30/2016 of the National Board of Control of Animal Experimentation (CONCEA).

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