



## Communication

[Comunicação]

### Does a single application of contraceptive cause pathological changes in bitches?

[Uma única aplicação de anticoncepcional causa alterações patológicas em cadelas?]

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The widely used form of reproductive control in domestic animals is pharmacological, mainly through the administration of progestins (Romagnoli, 2002). This form of contraception is characterized by veterinary contraceptives found in the form of injectable solutions or steroid hormone tablets that act by delaying or suppressing the sexual acceptance phase (Vigo *et al.*, 2011). Studies have shown a high frequency of complications observed after the use of progestins in dogs and cats, especially when applied in phases other than anestrus. The administration of these drugs leads to problems such as pyometra, fetal maceration, endometrial hyperplasia, mammary hyperplasia, mammary tumors (Araújo *et al.*, 2017), skin reactions at the application site, insulin resistance, among others (Romagnoli, 2002).

In view of the above, the present study aimed to evaluate the effects of administration in a single dose of progestins in healthy canine females who had never received such drugs. The project that originated this article was approved by the animal ethics committee of the Paranaense University under protocol number 34626/2018. Veterinary stores were visited and in these stores twenty female dog owners who acquired contraceptives to apply to their female dogs were approached. Thus, there was no interference from researchers in the acquisition of contraceptives. Only owners who were buying the product for the first time were selected. The data of the bitches selected for the project are listed in Table 1. Before the contraceptive application, all female dogs were

clinically examined. Anamnesis, physical examination, blood count, biochemical tests (total proteins, calcium, urea, creatinine and alanine aminotransferase), abdominal ultrasound and vaginal cytology were performed. Only bitches that did not show pathological changes in all exams were selected.

The female dogs were submitted to the contraceptive administration acquired by the owner (Table 1). All drugs were injectable and were applied subcutaneously to the right lateral abdominal region. Ninety days after the administration of contraceptives, all dogs were submitted to ovariohysterectomy by conventional method (Stone, 2007). The ovaries were identified in relation to the side and sectioned longitudinally. The uterine horns were opened in the longitudinal direction and identified in relation to the side. The pieces were then processed for histopathological exams. In the statistical analysis of the data obtained, a descriptive analysis of the data was performed by determining the absolute and relative frequency (%). For continuous variables, descriptive statistics were performed by determining the mean and standard deviation. The cross-reference table for the basal cytology and histopathological results used contraceptive and the result of histopathological analysis was performed. The results of the presence of animals with changes in histopathological examination (mild and severe cystic endometrial hyperplasia) for the phases of proestrus and estrus were compared using Fisher's Exact test.

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*Does a single application...*

Table 1. Data referring to the 20 female dogs included in a study about contraceptives

Breed	Age (month)	Weight kg	Drug	Cycle estrus phase by vaginal cytology	Uterine histopathological classification
German Shepherd	6	20	Anticion*	Anestrus	Normal
Chow Chow	11	18,8	Inibidex**	Estrus	MCEH
Mixed breed	26	8,55	Anticion	Estrus	MCEH
Mixed breed	24	9,2	Inibidex	Estrus	MCEH
Mixed breed	60	5	Inibidex	Proestro	SCEH
Mixed breed	24	7,8	Inibidex	Proestrus	NCC
Mixed breed	12	6,75	Inibidex	Diestrus	NCC
Mixed breed	60	5	Inibidex	Estrus	SCEH
Boxer	48	29	Inibidex	Estrus	MCEH
Mixed breed	6	9	Inibidex	Diestrus	NCC
Shih tzu	64	4,4	Anticion	Diestrus	NHCGE
Mixed breed	24	6,4	Inibidex	Proestrus	NHCEG
Daschund	29	6,3	Anticion	Proestrus	NHCEG
Mixed breed	24	7,6	Anticion	Proestrus	NCC
Shit Tzu	11	5,3	Inibidex	Proestrus	SCEH
Daschund	36	7	Anticion	Proestrus	NHCGE
Daschund	55	6,3	Anticion	Diestrus	NHCGE
Mixed breed	8	6,7	Anticion	Diestrus	NCC
Mixed breed	8	6	Inibidex	Proestrus	NCC
Pitt bull	108	29	Anticion	Proestrus	NCC

\* Anticion (2018) (Medroxyprogesterone acetate, UCBVET, Brazil)

\*\*Inibidex (2018) (Medroxyprogesterone acetate, LAVIZOO, Brazil)

MCEH: Mild Cystic Endometrial Hyperplasia

SCEH: Severe Cystic Endometrial Hyperplasia

NCC: Normal, with circulatory changes (edema and hemorrhage)

NHCGE: Normal with hyperplastic changes in the endometrial gland

For the red blood cells, leukocytes, platelets, urea, creatinine and alanine aminotransferase (ALT) variables over time (basal, 30 days and 90 days) the results were compared using Repeated Measurement Variance Analysis. The Mauchly sphericity test was used to assess the sphericity of the data. When the sphericity hypothesis was violated ( $P < 0.05$ ) the degrees of freedom were corrected using Greenhouse and Geisser's estimates of sphericity. The analyzes were performed using the statistic program IBM SPSS v. 21.0. For all tests, a significance level of 5% was considered. Only healthy bitches were selected for the project to prevent the application of the contraceptive from worsening any present disease. The use of contraceptives in dogs can only be considered safe if the animal is healthy at the time of administration (Romagnoli, 2006; Maenhoudt *et al.*, 2018). Among the diseases that can be aggravated by the use of contraceptives are diabetes, lesions or tumors in the mammary glands and cystic endometrial hyperplasia (Romagnoli, 2006).

When the bitches were examined again at thirty days, no changes in blood counts and ultrasound examinations were observed. However, on physical examination, 12 female dogs (60%; 12/20) had mammary hyperplasia (Figure 1). This change is due to the action of the progestin on the mammary gland. Progesterone induces important changes in the mammary gland, creating a highly proliferative environment inside the mammary gland (Martins and Lopes, 2005). The presence of mammary hyperplasia detected in the bitches in the study may be a predisposing factor for the appearance of mammary tumors. There is a report of an association between mammary tumors in female dogs and administration of contraceptives (Honório *et al.*, 2017). It was not possible to perform histopathology of the mammary glands because the owners did not authorize the biopsy it, which is a limiting factor of the present study.

In 18 bitches (90%; 18/20), the uterus presented macroscopic changes compatible with cystic endometrial hyperplasia (CEH) (Figure 2A). Of

these bitches that presented CEH, five presented purulent secretion in the uterus (27.77%; 5/18) in association with CEH (Figure 2B). The presence of pus associated with CEH characterizes pyometra (Kempisty *et al.*, 2013). Therefore, five healthy bitches presented pyometra with a single application of contraceptives. Upon microscopic examination, only one female dog had completely normal uterine tissue (Figure 3A), according to the classification proposed in the literature (Monteiro *et al.*, 2009). This bitch was in anestrus. The package inserts of the contraceptives used in the present study inform that the drug should be administered in the anestrus to minimize side effects (Anticion, no date; Inibidex, no date). However, the recognition of the bitch estrous cycle phase can only be performed by means of vaginal cytology, an examination restricted to the veterinarian (Silva, 2016).

On microscopic examination, 19 female dogs (95%; 19/20) presented uterine changes that ranged from circulatory changes (edema and hemorrhage) to severe cystic endometrial hyperplasia (Figure 3) (Table 1). Probably mild uterine changes (Figure 3) would tend to regress, and the animal could be normal in the next estrous cycle. The progesterone concentration in dogs with CEH is comparable to that observed during the diestrus of normal canine females (Chen *et al.*, 2001). However, three bitches presented severe CEH (Figure 3 B,C,D.) (Table 1), according to the literature classification (Monteiro *et al.*, 2009). Thus, it can be inferred that the application of the contraceptive promoted such changes, since only healthy bitches were selected for the study.

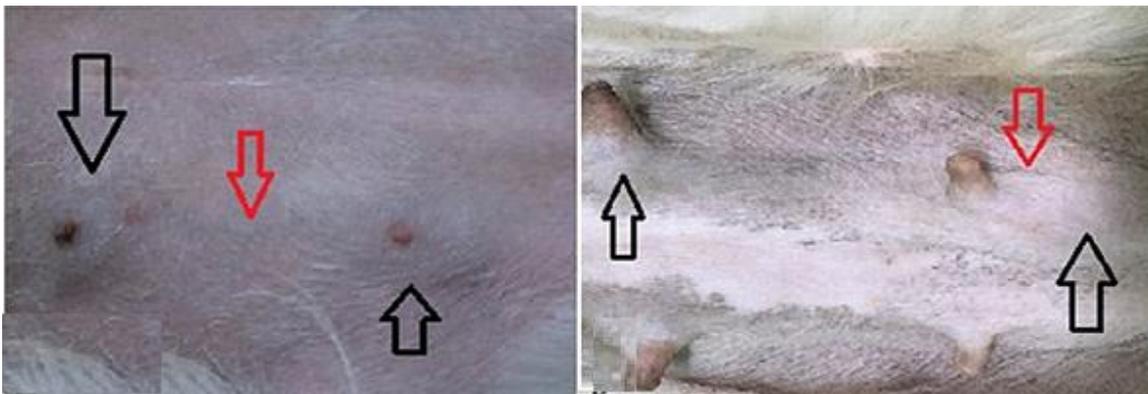


Figure 1. Mammary chain of bitches 30 days after being submitted to the application of injectable contraceptives. Black arrows: hypertrophied mammary glands. Red arrows: accentuated vascularization.

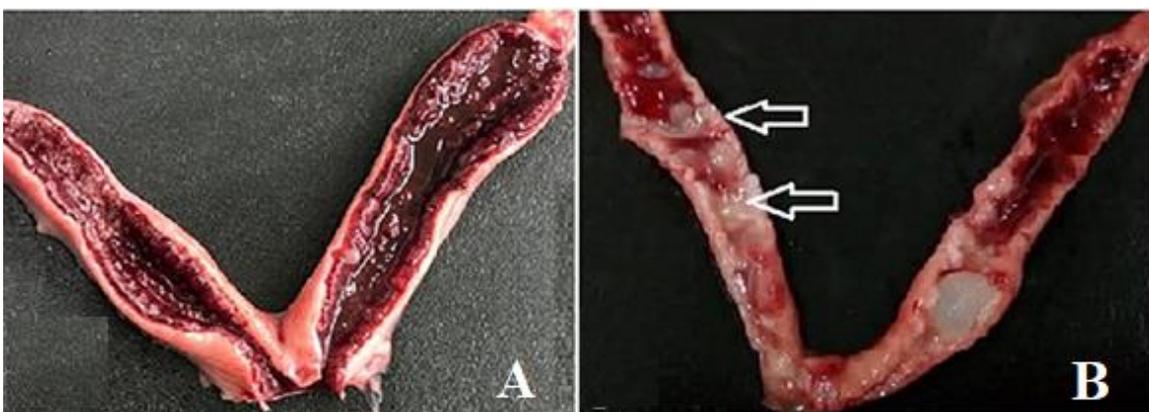


Figure 2. Uterus of bitches, ninety days after application of injectable contraceptive. A: Hemorrhagic and cystic areas are observed. B: There is the presence of purulent secretion (arrows).

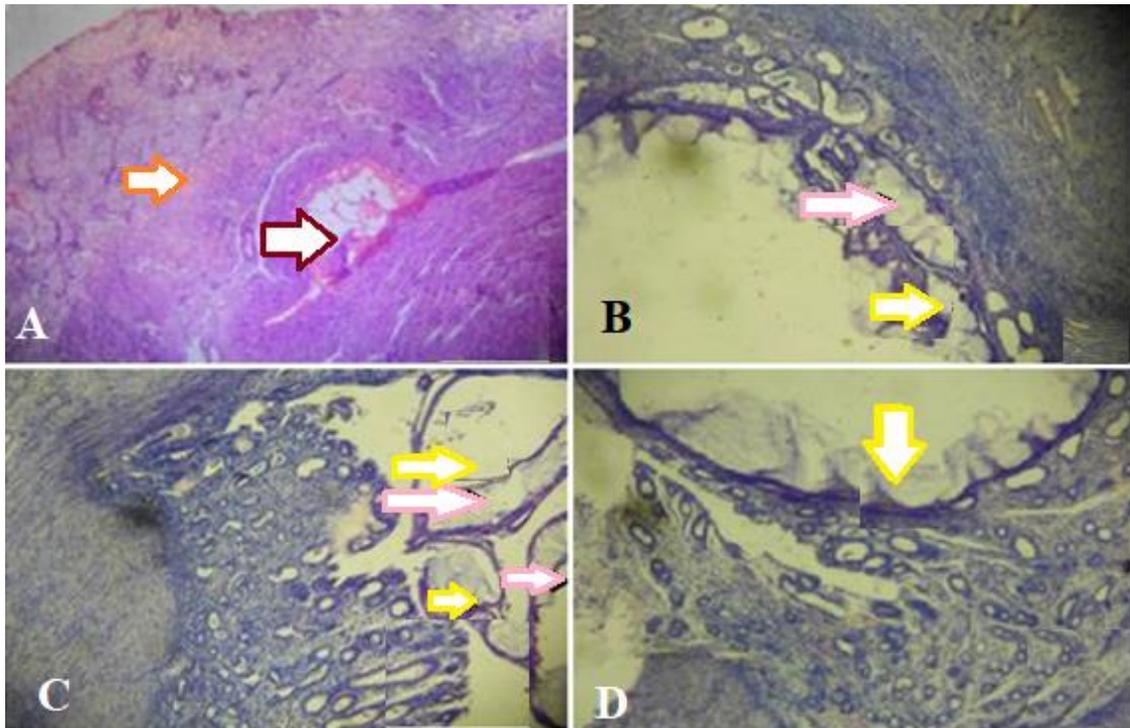


Figure 3. Histopathological section of bitch uterus, ninety days after the application of injectable contraceptives. A: The appearance is normal but shows circulatory changes (edema and hemorrhage). Observe vessel congestion (black/white arrow) and diffuse hemorrhage in the myometrium and endometrium (red/white arrow). B, C, D: Severe cystic endometrial hyperplasia. Cystic glands: yellow/white arrow. Content in the light of the glands: pink/white arrow). Photos captured in 4x / 0.10 lens.

Despite these results, statistical analyzes by Fischer's exact test comparing parameters two by two did not show statistical significance. It is likely that the absence of statistical significance is due to the small sample size compared to the various parameters analyzed. The administration of synthetic progestogens in bitches increases the number of estrogen and progesterone receptors in the endometrial glands, causing thickening of the endometrium. Such thickening is due to labyrinthic-like proliferations of surface epithelial cells, massive secretion, and cystic dilatation of

endometrial gland lumina all of which are characteristics of cystic endometrial hyperplasia (De Bosschere *et al.*, 2002). In this way, A single application of injectable contraceptives (package insert dose) in bitches was able to cause pathological changes in the reproductive system of these animals, mainly cystic endometrial hyperplasia.

*Keywords: canine, contraceptives, cystic endometrial hyperplasia, progestins*

## RESUMO

O presente trabalho objetivou avaliar os efeitos da administração em dose única de progestágenos em fêmeas caninas hígdas, as quais nunca haviam recebido tais fármacos. Foram selecionadas 20 cadelas, que foram examinadas clinicamente e por meio de exames complementares. Nessas cadelas, foi aplicado medroxiprogesterona por via subcutânea. Noventa dias após, as fêmeas foram esterilizadas cirurgicamente, sendo os tecidos reprodutivos encaminhados para histopatologia. Foi possível verificar que, aos 30 dias, 12 animais (60%) apresentaram hiperplasia mamária. Aos 90 dias, 18 animais (90%) apresentavam sinais de hiperplasia endometrial cística, tendo cinco (27,77%) destes animais apresentado conteúdo purulento no lúmen uterino. No exame microscópico, apenas uma fêmea não demonstrou alterações patológicas, sendo a única que recebeu o contraceptivo na fase correta (anestro). As demais fêmeas apresentaram alterações que variaram entre alterações circulatórias a hiperplasia endometrial

cística grave. Assim, foi possível concluir que uma única aplicação de anticoncepcional em fêmeas híginas pode causar complicações leves a graves.

Palavras-chave: canina, contraceptivos, hiperplasia endometrial cística, progesterágenos

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