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Histomorphometrics and quantitative unbiased stereology in canine uteri treated with medroxyprogesterone acetate

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ABSTRACT

This article describes the effects of MPA use on the canine uterus using stereological methods. Entire reproductive tracts were removed from normal healthy canine bitches (*Canis lupus familiaris*) and grouped as: nulliparous (n = 11), multiparous (n = 11) and MPA-treated (n = 11; nulliparous; two treatments; 5 mg/kg). 1 cm samples were cut from the corpus, horn and uterine tube and fixed in 10% formaldehyde. Sections of each were mounted on slides and stained with hematoxylin-eosin. We assessed the fraction area for components of endometrium and myometrium and V_V (volume density) and S_V (surface density) of the gland and stroma using the M_{36} test system provided by the STEPanizer Stereological Tool. No gross histological differences were observed between study groups in the uterine tube, uterine corpus and horn. The wall of the uterine corpus and horn in MPA-treated bitches was characterized as being thicker than in the other groups. A cross-section of the uterine corpus revealed no differences between components of uterine wall in the corpus and horn; however, differences were observed in the volume density [V_V; %] in variables such as: $V_{V[str.vasc/uterus]}$ (nulliparous *vs.* multiparous; $p = 0.0019$) and $V_{V[str.supravasc/uterus]}$ (multiparous *vs.* nulliparous and MPA; $p = 0.0035$). In the endometrial gland, differences were detected in $S_{V[gland/endom]}$ (multiparous *vs.* MPA, $p = 0.0442$). In the uterine horn, differences were only observed in the variable $V_{V[lumen.gland/endom]}$ (multiparous *vs.* MPA; $p = 0.0019$). This study shows quantitative changes in the architecture of the endometrium and myometrium in all the uterine segments, mainly morphological endometrial gland changes of the uterine corpus, increasing the surface area per unit of volume; however, these changes usually do not differ quantitatively from those observed in the uterus of multiparous bitches.

1. Introduction

Hundreds of dogs are euthanized annually under public health programs in order to control the overpopulation of stray dogs in urban centers. The usual method, surgical sterilization [1], prevents and reduces the frequency of uterine and mammary gland diseases. However, due to the high costs associated with performing a large number of surgical procedures and the possible postoperative complications, non-surgical methods have been proposed. One of these is treatment with medroxyprogesterone acetate (MPA), a long-acting synthetic progestin. In the United States, its use is not approved as a contraceptive treatment in dogs; however, its use in other countries to delay estrus is frequent. Although its effect is similar to luteal endogenous progesterone, a high incidence of side effects has been reported, including adrenocortical atrophy, diabetes mellitus, acromegaly and pyometra, among others [2–4]. In dogs treated with MPA for estrus suppression, the prevalence of clinical presentation of uterine and mammary disease is 45% [5]. It is estimated that the risks of MPA-related side effects are common and should be considered prior to the choice of surgical or non-surgical castration [1,6]. On the other hand, studies have reported normal morphology of the uterine tissue. For example, one study [7] evaluated the morphological and proliferative changes in the uterine tubes during anestrus, the luteal and follicular phases and the morphometric characteristics of the tubular epithelium. Changes in the endometrial epithelium have also been described in

Abbreviations: MPA, Acetate of Medroxyprogesterone; FSH, Follicle-stimulating hormone; LH, Luteinizing hormone; V_V, density volume; S_V, Surface density; SD, standard deviation.

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bitches in anestrus and metestrus [8] as well as the influence of steroid hormones on the histological characteristics of the uterus [9]. Previous research looking at MPA exposure in the female dog has focused on studying the long-term effects of MPA exposure as a means of controlling reproductive cyclicity in adult females [5], its effects on adenohypophyseal function [3] and pulsatile plasma profiles of follicle-stimulating hormone (FSH) and luteinizing hormone (LH) before and during MPA treatment [4]. However, there is insufficient information regarding the application of MPA during anestrus and its quantitative effects on uterine tissue.

The quantitative investigation of images taken from light microscopy observation is one of the pillars of biological and biomedical investigation. The introduction of stereology and design-based sampling in the biomedical field has been a major advance over the last 30 years [10]. The main advantage of this technique is that it can efficiently produce reliable and accurate results. Stereological estimates such as volume density (V_V) and surface (S_V) in uteri of bitches treated with MPA during anestrus have not been assessed previously; therefore, the interpretation of the results obtained here was difficult due to the absence of this subject in the literature.

It was hypothesized that intramuscular MPA treatment during anestrus would modify the uterine tissue and specifically would affect the development of components of the endometrium and myometrium as well as the morphology of the uterine tube. The purpose of this study was to develop a base of metric information regarding the effects of intramuscular MPA administration on the uterus and uterine tube. Specific objectives were to describe the quantitative histomorphological and unbiased stereological features in the uterus of female dogs exposed to the synthetic progestin MPA and to compare them with females exposed to physiological luteal progesterone under conditions of nulliparity and multiparity.

2. Materials and methods

This study was approved by the competent university review boards, and was conducted at the Center for Excellence in Surgical and Morphological Studies of the Universidad de La Frontera, Temuco, Chile.

2.1. Animals, samples and determination of estrus cycle

Complete uteri were obtained from 33 healthy adult female dogs (*Canis lupus familiaris*) from the University's Animal Hospital, with no defined breed, subjected to ovariohysterectomy during anestrus (18 months - 6 years). No gross abnormalities were present anywhere in these tracts. Uteri were classified as nulliparous (n = 11; older than 9 months, that had experienced at least one estrus cycle), multiparous (n = 11; older than 9 months, that had experienced at least two full-term gestations and involution of the uterus after the last gestation was complete) and MPA-treated bitches (n = 11; nulliparous exposed twice to contraceptive treatment during late anestrus). All the bitches included in this study demonstrated at least one estrus, with this information being determined from the owner's report and clinical records (Table 1). Treatment with a synthetic progestin depot preparation of MPA (OVO-6® 50 mg, Drag Pharma Laboratory Invetec S.A., Chile) was begun during anestrus giving two treatments of 5 mg/kg i.m. body weight at 8-week intervals. A vaginal inspection and cytological examination of the vaginal floor were performed on bitches used in this study to determine the estrus cycle and confirm the anestrus. Three days before the start of the treatment with MPA the stage of the estrus cycle was assessed. Anestrus was confirmed using physical examination, vaginal cytology (Diff Quick®, Hartman Leddon Co, Philadelphia, PA), histological parameters of the uterus and ovaries [11] and confirmed by the absence of the corpus luteum in the ovary. Vaginal cytology showed sparse numbers of parabasal cells and variable numbers of neutrophils. The vaginal mucosa appeared thin and red with visible capillaries; the surface was easily traumatized and vaginal cytology was difficult to monitor without inducing bleeding with spurious erythrocytes in smears classified as being in anestrus [12]. In terms of uterus histological parameters, bitches in anestrus had simple low columnar or cuboidal epithelium, superficial stroma rich in cells, and the stroma of the deeper part of the endometrium with less convoluted basal glands with simple cuboidal and in some cases columnar epithelium.

2.2. Uterus volume measurement and tissue preparation

The fresh uteri were separated from the ovaries and uterine tube. The broad ligament was dissected. The volume of each uterus was measured by the immersion method (Scherle 1970). Three 1 cm sections of each uterus (corpus and horn) and uterine tube (ampulla) were cut and fixed in 10% formaldehyde (Fig. 1A). The samples were processed to obtain paraplast sections for microscopic evaluation (Paraplast Plus embedding medium; melting point: 54 °C; Sigma-Aldrich Chemical Co., St Louis, MO, USA). The sections of uterus were processed through a series of alcohols with increasing concentration and cleared in xylene. The paraplast block was cooled and stored at 4 °C until sectioning. Sections (5 μm) deparaffinized and rehydrated through decreasing alcoholic solutions were mounted on slides. Sections of each sample were routinely stained with hematoxylin and eosin.

2.3. Morphometric, planimetric and stereological analysis

Digital photomicrographs of each tissue sample were obtained using a Leica®DM750 optical microscope equipped with a Leica®MC170HD digital camera. A histological evaluation at 40× was performed on uterine tube to describe the condition of the three groups evaluated. For histomorphometry the cross-histological sectional images of uterine tubes, horns and uterine corpora were measured using the AxioVision 4.7.1 software (Carl Zeiss, Jena, Germany). For planimetry, the fraction area of the uterine wall components (corpus and horn) was calculated by light microscopy at 1×, 2× and 4× magnifications (%; fraction area of endometrium, submucosal, vascular and supravascular layers). For stereology, sampling and acquisition of unbiased stereological estimators were performed. Serial histological sections (20 μm intervals) 5 μm thick were analyzed (corpus and horn); each stereological data was estimated by examining 5 microscopic fields per sample [13]. In the endometrium, the volume density (V_V; estimated by point counting) of the stromal tissue and lumen and glandular epithelium as well as surface densities (S_V; estimated by intersection counting) of the endometrial gland were calculated by light microscopy at 40× (Fig. 1B). The M_{36} test system provided by the STEPanizer Stereological Tool software [14] was used, which has 36 test points; the test line measures 18d and the test area measures 36.36d^2. The overlapping program analyzed images from the horns and uterine corpora. Total volumes of glandular epithelium, lumen and stroma within the endometrium of each uterus were determined by multiplying the mean proportion of each tissue within each uterus by the total volume of each respective uterus [15]. All stereological and planimetric evaluations considered the tissue deformation [16]. All measurements were performed by two trained observers, each using his own microscope and computer software, blinded to the identity of the groups.

Table 1
Age (years), number of estrous cycles and period since the last parturition (time PP) in studied bitches (n = 11 *per group*).

Nulliparous		Multiparous			MPA-treated	
Age (years)	Estrous Cycle	Age (years)	Estrous Cycle	Time PP (month)	Age (years)	Estrous Cycle
3.5	4	3.0	4	12	3.0	3
3.0	4	3.0	4	14	3.0	4
3.0	4	3.0	3	12	3.0	4
3.0	3	3.0	3	12	3.0	2
1.5	2	5.5	4	14	3.0	3
2.0	3	3.0	3	14	2.0	2
3.0	3	3.5	3	14	2.0	3
3.0	4	3.5	3	12	2.5	3
3.0	3	3.0	4	12	3.0	3
3.0	4	3.0	3	14	3.0	3
3.0	4	3.0	4	14	3.0	5

2.4. Statistical analyses

Data were expressed as mean ± standard deviation (SD). The D'Agostino-Pearson test was used to evaluate data normality. Data for uterine volume, volumes of uterine tissue components and volumes of endometrial tissue components were analyzed by one-way ANOVA among the three groups and Tukey's post-test of multiple comparisons was used. Statistical significance for all of hypotheses tests was set at $p < 0.05$. The data analysis was performed using GraphPad Prism 5.0 software for Mac OS X (GraphPad Software, San Diego CA).

3. Results

The age (years) of the dogs used in our study, in the Nulliparous, Multiparous and MPA-treated groups, was 2.8 ± 0.6, 3.3 ± 0.8 and 2.9 ± 0.4, respectively ($p = 0.0517$). The number of estrous cycles the bitches experienced prior to the study, in the Nulliparous, Multiparous and MPA-treated groups, was 3.4 ± 0.68, 3.4 ± 0.52 and 3.1 ± 0.8, respectively ($p = 0.4720$).

3.1. Histology of the uterine tube (ampulla)

No gross histological differences were observed among the three study groups. In the tunica mucosa, the three groups had irregular primary, secondary and tertiary folds separated by wide spaces, covered by an epithelium that varied from simple cuboidal to columnar with secretory cells. The ampulla presented a mucosa with a large number of branched folds, and in some cases, they even occupied a large part of the lumen. Also, loose collagenous connective tissue was observed with slight hyperemia, associated with the presence blood vessels. The *tunica muscularis*, presented irregular compact collagenous connective tissue and discrete blood vessels were observed between the circular and longitudinal layers. However, MPA-treated bitches had a loss of cilia in the apical region in the tunica mucosae and presented some debris and macrophages in the lumen. In addition, collagen fibers and small fatty cells were observed between the tunica mucosa and tunica muscularis. The longitudinal layer presented various smooth muscle fascicles cross-sectionally and these were separated by loose collagenous connective tissue (Fig. 2).

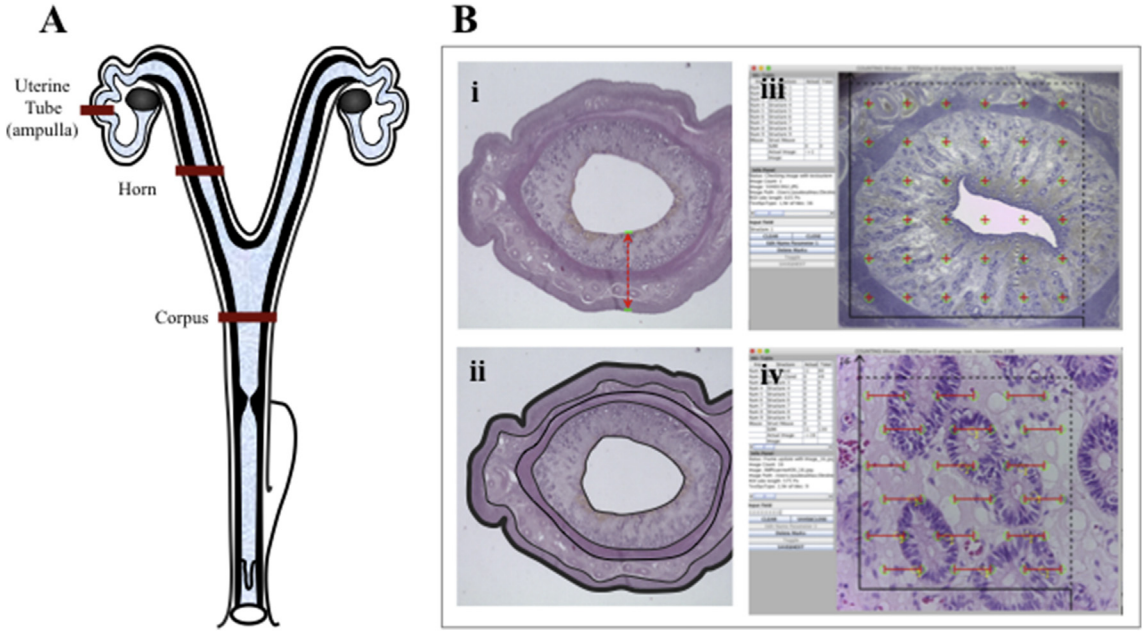

Fig. 1. A) Canine uteri. Demonstrates the sampling site that was studied. B) Interface example used in i) histomorphometric (1×) and ii) planimetric studies (1×). Also, interface example used in iii) planimetric (10×) and iv) stereological (40×) study using M_{36} test system provided by the STEPanizer Stereological Tool software.

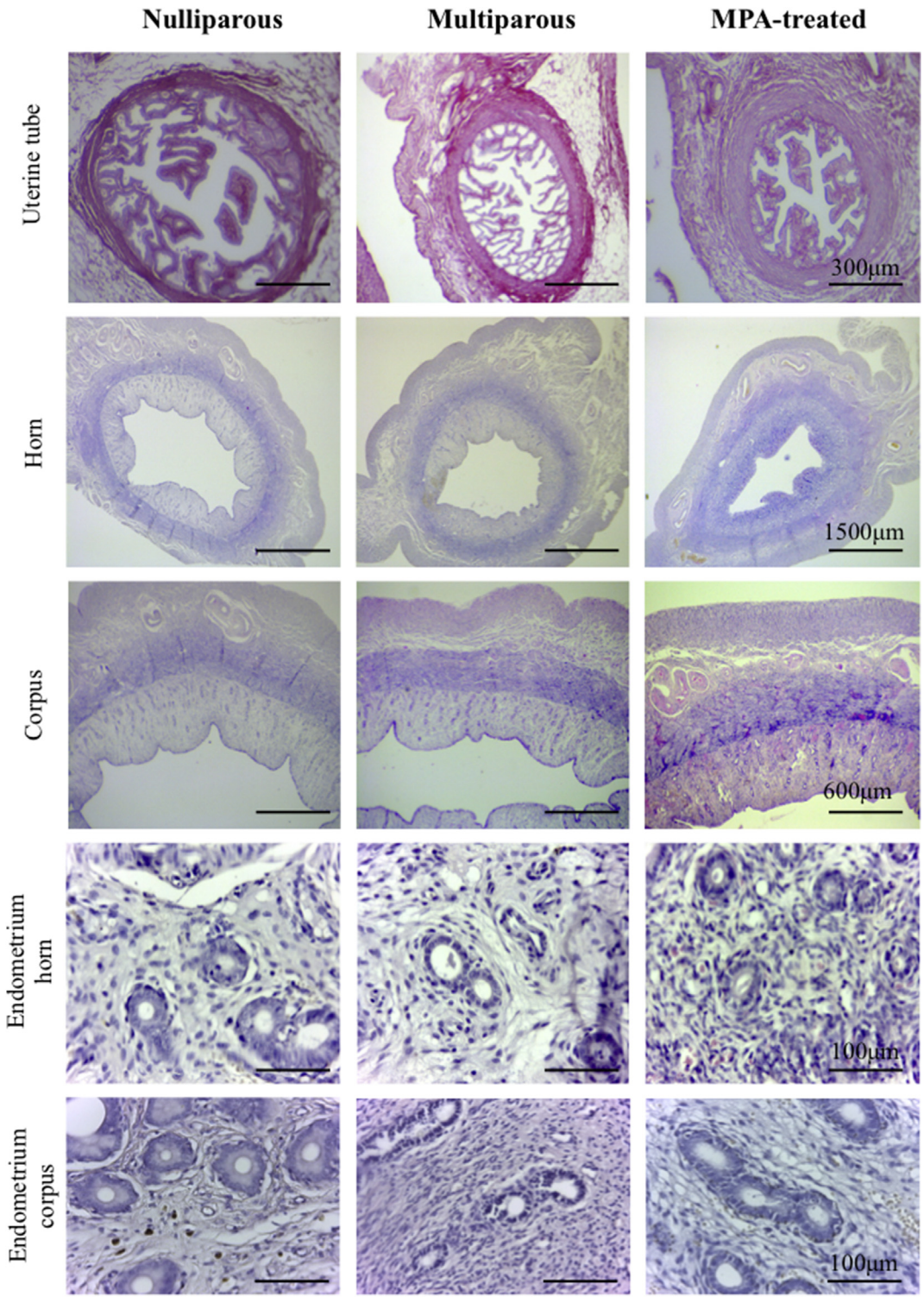

Fig. 2. Photomicrograph showing typical histological appearance of uterine tissue section in different segment in nulliparous, multiparous and MPA-treated bitches. Hematoxylin-eosin.

3.2. Histomorphometry and planimetry

In general, multiparous and MPA-treated uteri presented a greater thickness of the uterine wall. Moreover, no differences were observed among groups in the total thickness in the uterine tube. In the uterine corpus differences in the total thickness were observed between multiparous and MPA-treated *vs*. nulliparous ($p = 0.0274$). MPA-treated bitches presented almost double the uterine thickness of nulliparous bitches. In the uterine horn, a difference in the total thickness was observed between multiparous and MPA-treated *vs*. nulliparous. The histomorphometric measurements in the corpus, horn and uterine tube in 11 uteri are summarized by group in Table 2. In the fraction area (%) of the uterine wall components, no differences were observed between the corpus and horn (Fig. 3).

Table 2
Histomorphometric parameters (μm; mean ± SD) in uteri of nulliparous, multiparous and MPA-treated bitches (*h* = height).

	Nulliparous (n = 11)	Multiparous (n = 11)	MPA-treated (n = 11)	*p-value*
Uterine tube (ampulla)				
Total thickness	814.5 ± 98.1	901.2 ± 101.4	893.0 ± 99.3	0.0522
Tunica mucosa h	207.5 ± 45.5	206.6 ± 122.0	205.8 ± 40.3	0.9991
Tunica muscularis				
Circular layer *h*	365.8 ± 80.8	348.8 ± 175.1	380 ± 88.8	0.6349
Longitudinal layer *h*	332.9 ± 41.1[a]	250.0 ± 94.7[b]	261.4 ± 60.3[b]	<0.0001
Corpus				
Total thickness *h*	765.0 ± 101.2[a]	1311.9 ± 156.8[b]	1496.0 ± 218.3[b]	0.0274
Endometrium	415.1 ± 296.0[a]	147.5 ± 92.46[b]	386.6 ± 248.3[a]	<0.0001
Glandular diameter	67.7 ± 37.2	68.8 ± 22.48	62.4 ± 19.6	0.4753
Glandular lumen diameter	28.4 ± 20.4[a]	65.1 ± 22.16[b]	33.4 ± 15.3[a]	<0.0001
Myometrium				
Submucosal layer *h*	188.8 ± 78.7[a]	439.9 ± 276.7[b]	526.4 ± 190.0[b]	<0.0001
Vascular layer *h*	255.9 ± 125.2[a]	410.4 ± 167.8[b]	566.0 ± 385.2[b]	0.0002
Supravascular layer *h*	288.0 ± 148.3[a]	417.3 ± 217.9[b]	442.2 ± 128.9[b]	0.0039
Uterine horn				
Total thickness *h*	954.7 ± 22.0[a]	1287.0 ± 255.8[b]	1106.6 ± 109.0[b]	0.0404
Endometrium	381.8 ± 170.4[a]	218.1 ± 128.7[a]	609.2 ± 817.3[b]	0.0002
Glandular diameter	79.9 ± 83.1	60.3 ± 25.3	64.1 ± 24.8	0.0725
Glandular lumen diameter	30.9 ± 32.6	63.4 ± 26.0	31.8 ± 17.8	<0.0001
Myometrium				
Submucosal layer *h*	247.1 ± 184.1[a]	344.4 ± 168.4[b]	340.3 ± 68.6[b]	0.0156
Vascular layer *h*	310.9 ± 209.0	429.9 ± 241.8	449.4 ± 206.3	0.0854
Supravascular layer *h*	367.7 ± 208.2[a]	454.2 ± 123.1[b]	252.0 ± 84.6[a]	0.0003

*letters indicate statistical differences between groups ($p < 0.05$).

3.3. Stereology

Stereological estimators and absolute data of wall components of the uterine corpus and horn are summarized in Table 3, presented as the mean ± SD. A cross-section of the uterine corpus revealed differences in the volume density [V_V; %] in variables such as $V_{V[str.vasc/uterus]}$ (nulliparous *vs*. multiparous; $p = 0.0019$) and $V_{V[str.supravasc/uterus]}$ (multiparous *vs*. nulliparous and MPA; $p = 0.0035$). In the endometrial gland, differences were detected in $S_{V[gland/endom]}$ (multiparous *vs*. MPA, $p = 0.0442$). In the uterine horn, differences were only observed in the variable $V_{V[lumen.gland/endom]}$ (multiparous *vs*. MPA; $p = 0.0019$).

4. Discussion

To our knowledge, this is the first study reporting quantitative morphological effects on uteri attributable to the application of MPA as a contraceptive treatment in bitches using morphometric, planimetric and unbiased stereological methods.

The wall of the ampulla of uterine tube in the three groups

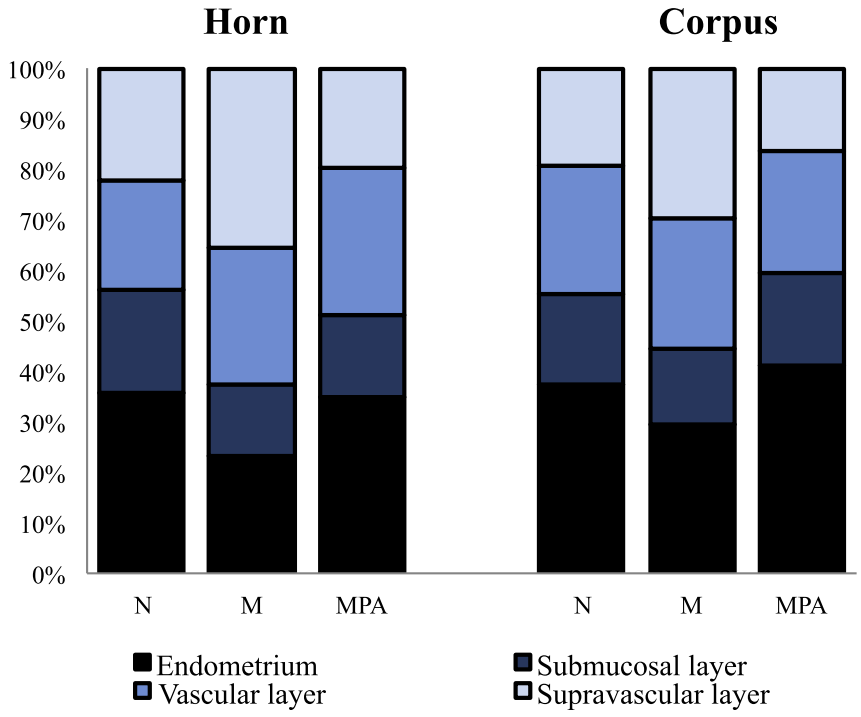

Fig. 3. Stacked bar graph depicting mean fraction area of various uterine tissue components for nulliparous (N), multiparous (M) and MPA-treated (MPA) bitches.

Table 3
Quantitative unbiased stereology estimates on uterine wall (mean ± SD).

	Nulliparous (n = 11)	Multiparous (n = 11)	MPA (n = 11)	*p-value*
$V_{(uterus)}$ [cm^3]	9.6	15.7	11.9	
Uterine corpus				
$V_{V(endom/uterus)}$ [%]	32.28 ± 8.95	28.25 ± 11.10	25.54 ± 14.63	0.0642
$V_{V(str.submuc/uterus)}$ [%]	19.40 ± 10.41	16.66 ± 6.20	17.34 ± 6.66	0.5834
$V_{V(str.vasc/uterus)}$ [%]	20.74 ± 11.33a	32.76 ± 15.86b	30.63 ± 11.19b	0.0019
$V_{V(str.supravasc/uterus)}$ [%]	21.35 ± 14.62a	34.37 ± 15.57b	20.71 ± 8.40a	0.0035
$V_{V(stroma/endom)}$ [%]	66.00 ± 14.52	67.30 ± 14.29	61.83 ± 16.03	0.4584
$V_{V(epith.gland/endom)}$ [%]	26.63 ± 11.87	26.66 ± 11.66	33.18 ± 15.54	0.1751
$V_{V(lumen.gland/endom)}$ [%]	7.32 ± 5.21	6.04 ± 4.76	5.00 ± 5.11	0.1581
$S_{V(gland/endom)}$ [mm^2/mm^3]	14.15 ± 8.91[a]	11.42 ± 5.59[b]	17.10 ± 8.62[b]	0.0442
Uterine horn				
$V_{V(endom/uterus)}$ [%]	35.01 ± 14.95	30.41 ± 11.00	41.13 ± 17.43	0.1116
$V_{V(str.submuc/uterus)}$ [%]	16.78 ± 5.32	15.24 ± 5.97	18.42 ± 5.21	0.2422
$V_{V(str.vasc/uterus)}$ [%]	23.57 ± 10.32	26.54 ± 9.61	24.05 ± 13.57	0.7183
$V_{V(str.supravasc/uterus)}$ [%]	18.18 ± 8.18	20.64 ± 4.62	10.46 ± 8.41	0.0543
$V_{V(stroma/endom)}$ [%]	62.42 ± 15.71	59.67 ± 23.51	57.95 ± 18.28	0.5984
$V_{V(epith.gland/endom)}$ [%]	30.06 ± 13.51	28.98 ± 16.78	37.53 ± 17.94	0.1579
$V_{V(lumen.gland/endom)}$ [%]	7.53 ± 6.80[a,b]	12.35 ± 10.24[a]	4.52 ± 4.83[b]	0.0019
$S_{V(gland/endom)}$ [mm^2/mm^3]	15.81 ± 8.86	13.37 ± 9.20	16.16 ± 8.05	0.541

evaluated did not present any qualitative or quantitative differences. The metric records obtained in this study seem to indicate that the application of MPA as a contraceptive does not affect the overall thickness of the wall of the uterine tube. The components observed in this segment and their distribution in the nulliparous, multiparous and MPA-treated groups were consistent with what has been described in the literature [7], and there were no differences among them. Nulliparous bitches presented a higher and more developed longitudinal layer than the other groups. The absence of mechanical stress on the uterine wall induced by the presence of viable fetuses in nulliparous females and the apposition of membranes and endometrial repair processes in multiparous females could explain these effects. In addition, it is known that non-pregnant *vs*. pregnant bitches present differences during the luteal phase in their respective secretion patterns [17,18], particularly in pregnant dogs that showed variability in progesterone synthesis and the duration of the luteal phase, suggesting bitches with a history of being exposed to different progesterone concentrations among them. It is therefore unlikely that a unique progesterone secretion pattern can be applied to all the dogs (multiparous and nulliparous). Hence, with respect to the concentration of progesterone during the luteal phase, the multiparous dogs would be exposed to varying progesterone concentrations, unlike nulliparous dogs that present less variability and a more homogeneous exposure. The variability in serum progesterone concentrations during the luteal phase could explain the effects observed afterward and in the medium term during anestrus.

It has been reported that the differences in the thickness of both muscle layers in the tube and uterus are due to the circular layer permanently contracting; however, the longitudinal layer is kept under a progestational block, and only when the progesterone levels drop is there a normal increase in uterine motor activity as a result of structural and functional development [19]. Apparently, MPA does not interfere with this mechanism in anestrus bitches. By contrast, other theories have asserted that there are different degrees of receptivity to ovarian steroidal hormones and synthetic progestins in the tubal tissue, and therefore the effect on the muscle layer is different depending on the estrus cycle [20] and possibly on the use, dose and dosage of MPA or another progestin. A similar report describes a decrease in thickness and consequently contractility of the fibers in the muscle layers of the infundibulum [7]. Our results suggest that administration of 5 mg/kg of MPA i.m. during anestrus would not exert morphological effects on the uterine tube different from those observed in untreated bitches.

In general, the wall of uterine corpus and horn in MPA-treated bitches was characterized as being thicker than in the other groups. These metrics demonstrate that the application of MPA as a contraceptive treatment promotes changes in the thickness of the uterine wall in these segments. The histological characteristics observed in the uterine segments in the three study groups are consistent with what has been reported in the literature [2,7,21].

The endometrial morphological changes in the uterine corpus and horn in dogs are influenced by hormonal stimuli [22]; nevertheless, it has been described that these do not occur with the same magnitude in the different segments of the uterus, probably due to a difference in the hormonal effects on the surface of the endometrial epithelium and the lamina propria [23]. In our study, this situation was not observed, since the three groups presented the same proportion of components of the uterine wall in the horn and the corpus (Fig. 2). Regarding the endometrial glands, the treatment with MPA during anestrus seems to maintain a state of glandular atrophy in both uterine segments similar to that observed in nulliparous bitches, associated with a relatively low glandular surface density (Table 3). This type of histological pattern has been described in other species, likes mares in anestrus, and has been related to progestational effects [24]. In the corpus and horn, no differences were observed between MPA-treated and multiparous bitches in the diameter of the glandular lumen, which can probably be attributed to glandular epithelial hyperplasia in bitches exposed to luteal progesterone during successive pregnancies and to the effect of treatment with MPA on glandular epithelium thickness during anestrus [9]. Thus, we can suggest the differences detected between groups in uterine endometrial glands would be attributable to administration of MPA and its effects on the tissue architecture. The evidence obtained in this study indicates that the morphological effect of the intramuscular administration of MPA on the wall of the uterus is similar to that observed one year postpartum; therefore and according to our results, both situations influence the morphological and morphometric characteristics of the submucosal, vascular and supravascular layers. It should be noted that one study described these same effects in dogs treated with MPA for 12 weeks [25]. According to our results, we suggest that luteal or exogenous progesterone-like MPA plays a significant role in the development of the muscle layers; however,

this effect apparently exerts a greater influence on the uterine corpus and horn in multiparous and MPA-treated bitches, possibly due to the greater number of progesterone receptors [26,27]. This suggests a biochemical effect that delays or stops the development of glands in the endometrium without altering myometrial development and growth.

The S_V and V_V of uterine structures were measured to determine the effects of contraceptive treatment with MPA. There are indications in uterine corpus measurements that the vascular layer and endometrial glands surface predominate. Moreover, in the uterine horn, the glandular lumen decreased. This suggests that MPA has the ability to induce angiogenesis when the administration method is systemic [28]. However, this situation does not occur in the uterine horn, perhaps due to the increase in diameter of the blood vessels from the uterine artery related to the uterine corpus region. The measurements in the components of the endometrium (stroma, epithelium and lumen glandular) of the uterine corpus indicate that although no difference was observed between the study groups, it should be pointed out that the MPA-treated uteri presented the lowest measurements in terms of the $V_{V\ gland.lumen/endom}$. This suggests a possible effect of luteal progesterone associated with glandular atrophy that would ultimately influence glandular volume density [9], which was also observed in the histomorphometric measurements. With respect to the $S_{V(gland/endom)}$, there were no differences between uteri of MPA-treated and nulliparous bitches. In the corpus and horn the glandular $S_{V(gland/endom)}$ in the MPA group was greater than uteri in nulliparous and multiparous bitches, suggesting an increase in tortuosity and branching of the glands under the influence of MPA. It has been widely reported that the effects of synthetic progesterone are similar to the endogenous kind [25]. However, and according to our results, in general, apparently different morphological characteristics were observed between MPA-treated uteri and those exposed to endogenous luteal progesterone. It is possible that the use of synthetic progesterone produced atypical morphological changes in the endometrium and myometrium.

We observed a link between the histomorphometric and stereological study, which means that the histomorphometric linear measurements seem to be important to establishing differences between the uterine tissue among the study groups. On the other hand, the stereological measurements showed a high correlation in the histomorphometric description between uterine tissues exposed to luteal progesterone and MPA [29,30]. Finally, it is worthy of note that this study has a limitation to consider: when MPA is used to suppress estrus in dogs, a single simple dose of 2 mg/kg should be administered during anestrus one month prior to the follicular phase [12]. In this study, the doses and frequency of application were greater (5 mg/kg, 2 doses); consequently, we cannot infer that lower doses will result in different outcomes. In conclusion, although it has been shown that MPA treatment affects ovulation, estrus and the later luteal phase, this study shows quantitative changes in the architecture of the endometrium and myometrium in all the uterine segments, mainly morphological endometrial gland changes of the uterine corpus, increasing the surface area per unit of volume. However, these changes usually do not differ quantitatively from those observed in the uterus of multiparous bitches. Finally, this study provides insight on the use of MPA. The information is useful for breeders and veterinarians who make decisions particularly regarding problems of contraception in bitches.

Conflict of interest statement

None of the authors has any conflict of interest to declare.

Author contributions

All the authors collaborated in the writing and bibliographic review. Paulo Salinas coordinated the work.

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