Histomorphologic and morphometric evaluation of the uterine horns in nulliparous and multiparous Beagles

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Objective—To macroscopically, histomorphologically, and morphometrically compare uterine tissues obtained from nulliparous and multiparous dogs.

Animals—12 dogs constituting 2 homogenous groups (7 nulliparous Beagles and 5 multiparous Beagles, each of which had at least 7 pregnancies).

Procedure—Serum concentrations of progesterone and estradiol-17β were determined. Samples of uterine tissues were fixed, embedded in paraffin, and cut into serial cross sections. Morphometric analysis was performed on systematically selected trichrome-stained sections.

Results—Mean absolute volume of the uterine wall did not differ between groups. Volume of blood vessels was significantly higher and relative mean value for myometrial connective tissue was significantly lower in the multiparous dogs. Arteries in the myometrium of multiparous dogs had pronounced thickening of the intima (ie, pregnancy sclerosis) and a concomitant thinning of the smooth muscle layer (tunica media). Furthermore, the elastica interna of these arteries appeared to be disintegrating and was highly and irregularly folded. Adventitia of the myometrial veins in multiparous dogs contained numerous layers of elastic fibers; however, only a few layers were observed in the adventitia of corresponding veins in nulliparous dogs.

Conclusions and Clinical Relevance—In this study, we documented that in contrast to other species, the uterus of nonpregnant dogs does not increase in size and volume even after at least 7 pregnancies. Furthermore, sclerotic alterations of uterine arteries are proof of at least 1 pregnancy. Results of this study may be useful in the evaluation of uterine diseases. (*Am J Vet Res* 2004;65:552–558)

The uterine horns are composed of the endometrium, myometrium, and perimetrium. The propria of the endometrium contains the uterine glands. The myometrium encloses the stratum vasculare with large uterine blood vessels between its inner circular and outer longitudinal layers of smooth muscle. During pregnancy, the uterus increases in size to accommodate the developing fetuses. This increase in weight, diameter, and length is mainly accounted for through hypertrophy and hyperplasia of uterine tissues.[1-7] Immediately after parturition, the reverse process of postpartum involution begins and is accompanied by a reduction in size, degradation, and loss of uterine tissue components (particularly collagen).[5,8-10] Comparison among domestic mammals reveals that involution is most rapid in mares and sows and least rapid in bitches, in which this process is not completed until 12 weeks after parturition.[9,11] Pregnancy may cause lasting quantitative and qualitative changes in the various uterine tissues.[2,12,13,a]

To our knowledge, there is only 1 morphologic and morphometric report[b] comparing the uteri of nulliparous and multiparous dogs. In that study, thickness of various uterine tissue layers was measured in a 2.5-month-old Fox Terrier, a nulliparous 2.5-year-old Bulldog, and an adult multiparous Dachshund. The objective of the study reported here was to provide detailed morphometric and morphologic data from uteri of nulliparous and multiparous bitches by use of homogenous groups of Beagles to provide insights into the lasting effects of gestation on uterine tissues.

Materials and Methods

Animals—Two groups of Beagles (7 nulliparous and 5 multiparous) in anestrus or late metestrus that weighed between 8.5 and 11.3 kg were used in the study. Nulliparous dogs were between 20 and 27 months old, whereas multiparous dogs were between 8 and 9 years old. Each multiparous bitch had given birth to at least 7 litters, and involution of the uterus after the last gestation was complete.

Procedure—Blood samples were collected for use in determining serum concentrations of progesterone and estradiol-17β for assessment of the stage of the reproductive cycle. Dogs were then euthanatized by IV administration of an overdose of sodium pentobarbital. Concentrations of progesterone and estradiol were measured without extraction by use of solid-phase radioimmunoassays.[c,d]

Collection and preparation of tissue samples—The uterus and cervix of each dog were removed through an incision in the linea alba and inspected macroscopically. A transverse section (1.5 cm in length) was excised from the middle of each uterine horn. In addition, smaller samples were collected from the cranial and caudal regions of the uterus for comparison. Tissue specimens were fixed by immersion in 3.7% paraformaldehyde in 0.1M calcium acetate (1.45 Osm; pH, 7.4) for 24 hours at 5°C. The fixed specimens were routinely embedded in paraffin and cut into serial cross sections that were 5 to 7 µm thick. For morphometric analysis, selected serial sections were stained with Gomori trichrome stain[14] to enable us to clearly differentiate between smooth muscle and connective tissue fibers. In addition, paraffin sections were stained with orcein to enable us to clearly see elastic fibers.[15]

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Variables—Several target variables were estimated, including the **reference volume** (**Vref**; volume of the uterine wall), relative **compartment volume** (**Vcomp**), and relative **structure volume** (**Vstructure**). The Vcomp included volume of the endometrium in relation to Vref and volume of the myometrium (including the perimetrium) in relation to Vref. The relative Vstructure included volume of the uterine glands (including the lumina) in relation to volume of the endometrium, the remaining endometrial tissue (connective tissue) in relation to volume of the endometrium, volume of the smooth muscle in relation to volume of the myometrium (including the perimetrium), volume of the connective tissue in relation to the myometrium (including the perimetrium), and volume of the blood vessels (including the lumina) in relation to the myometrium (including the perimetrium). Values for Vcomp were expressed as Vcomp = (Vcomp/Vref) × Vref, whereas values for Vstructure were expressed as Vstructure = (Vstructure/Vcomp) × Vcomp.

Morphometric analysis—The morphometric (stereologic) procedure has been described in detail elsewhere.[16,17] Briefly, the aforementioned target variables were estimated from results of 3 parallel serial sections (3.33 mm apart) of a 1-cm-long sample from the middle of the uterine horns, whereby the position of the first section was randomly selected. Values for Vref and Vcomp were estimated[19] in accordance with the Cavalieri principle. Estimted values for Vref (or Vcomp) were calculated by use of the following equation:

$$\text{estimated Vref (or Vcomp)} = \text{total cross-sectional area} \times \text{distance between sections}$$

Total cross-sectional areas of the uterine wall and its compartments were estimated by use of point counting.[19] Serial sections were analyzed by use of an image-analysis system[e] comprising a microscope[f] fitted with a camera.[g] Direct images from the sections were viewed on a monitor[h] at a final microscopic magnification of 16×. A grid (distance between grid lines, 300 µm) was superimposed on these images; points of intersection were used as a test system with a distance of 300 µm between test points. Therefore, the unbiased volume estimator was determined by use of the following equation:

$$\text{estimated Vref (or Vcomp)} = \text{object length} \times d^2 \times 0.333 \times \Sigma P_i$$

where d is distance (ie, 300 µm), and ΣP_i denotes the total number of test points counted in the uterine wall and its compartments. Volume fractions of the compartments were obtained in the same manner by use of the following equation:

$$\text{estimated Vcomp/Vref} = \Sigma P_i(\text{comp})/\Sigma P_i(\text{ref})$$

To estimate volume fractions of structures in the corresponding compartments (ie, Vstructure/Vcomp), the same sections were examined at a microscopic magnification of 160×. Each section was subsampled systematically (5 to 12 microscopic fields/section). A test system consisting of 2 sets of points with a ratio of numerical densities of 1:4 was placed over these fields. The corresponding distances were 600 µm (myometrium or endometrium) and 300 µm (smooth muscle, connective tissue, blood vessels, uterine glands, or remainder of the endometrium). The required volume fractions were estimated by use of the following equation:

$$\text{estimated Vstructure/Vcomp} = 0.25 \times (\Sigma P_i[\text{structure}]/\Sigma P_i[\text{comp}])$$

Criteria for design and allocation of test points were based on efficiency considerations.[19] A minimum of 200 points was counted in each relevant structure. Absolute volumes of the relevant structures were computed by use of the following equations:

$$\text{Vcomp} = (\text{Vcomp/Vref}) \times \text{Vref}$$
$$\text{Vstructure} = (\text{Vstructure/Vcomp}) \times \text{Vcomp}$$

To evaluate the possible influence of multiple pregnancies on thickness of the wall of myometrial arteries, measurements were made on arteries in the stratum vasculare of the uterus of each nulliparous and multiparous bitch. At least 7 cross sections of myometrial arteries with an external diameter of 100 to 200 µm were selected for measurement in each dog. Thickness of vessel walls (media and intima) and external diameter in the same location were measured by use of the aforementioned image-analysis system.[e] Subsequently, the ratio of mean wall thickness to external diameter was computed and analyzed.

Statistical analysis—Mean ± SEM values were computed for all morphometric data. Data were assessed for normal distribution and subjected to a factorial ANOVA by use of statistical software[i] to evaluate the significance of pregnancy-related differences. Hormone concentrations and age of the dogs were included as covariants. Significance was set at values of $P < 0.05$.

Results

Macroscopic and microscopic anatomy—We did not detect evidence of pathologic changes in the excised uteri. Uterine horns were 10 to 12 cm in length, and their external diameter varied between 5 and 11 mm. We did not detect differences between nulliparous and multiparous bitches. Clinical examination, serum hormone concentrations, and histologic results revealed that 2 nulliparous dogs and 1 multiparous dog were in anestrus; all other bitches were in late metestrus.[20]

Cross sections of uterine horns were circular to oval. Shape and width of the lumen varied with the thickness and folding of the mucosa. The endometrium was clearly distinct from the myometrium (**Fig 1**).

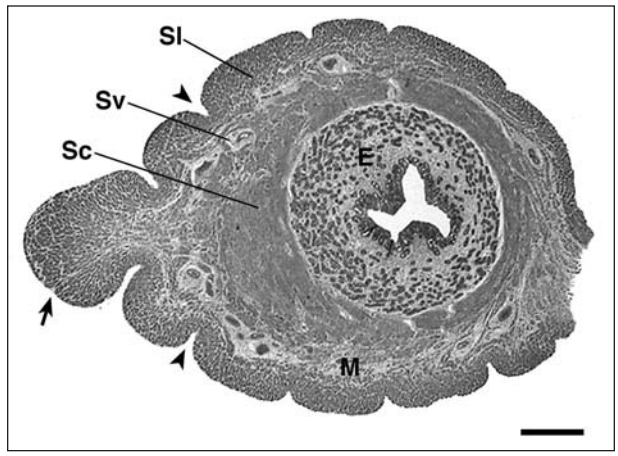
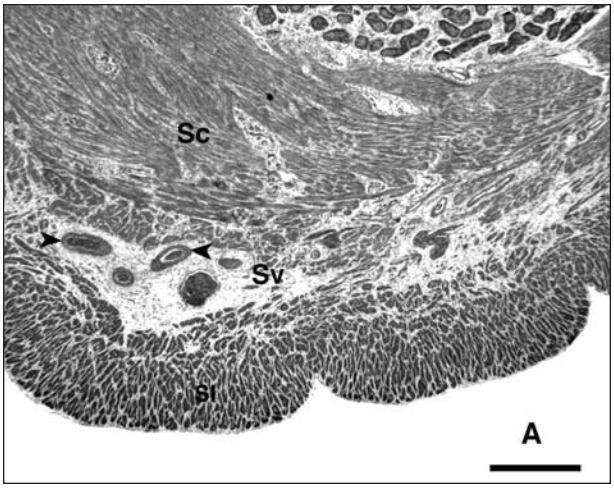


Figure 1—Photomicrograph of a cross-sectional view of uterine tissue obtained from the middle of the uterine horn of a 2-year-old nulliparous Beagle. Notice the bulge in the antimesometrial surface (arrow) and several superficial indentations (arrowheads). M = Myometrium. Sl = Stratum longitudinale. Sv = Stratum vasculare. Sc = Stratum circulare. E = Endometrium with secretory units of the uterine glands. Gomori trichrome stain; bar = 2 mm.



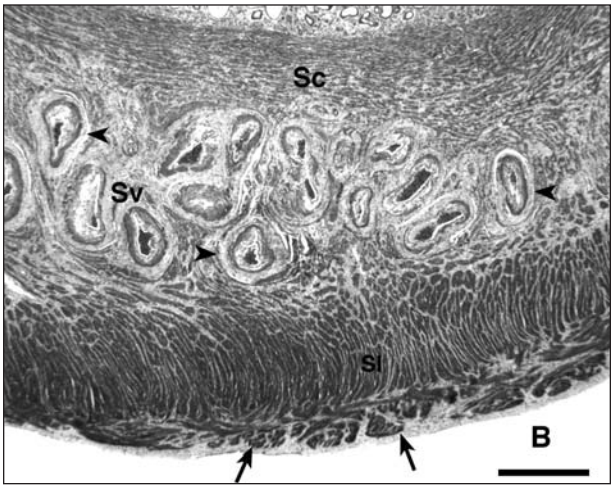
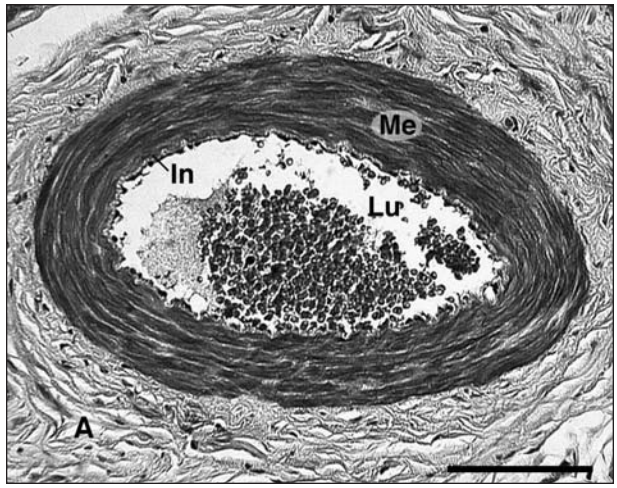


Figure 2—Photomicrographs of a cross-sectional view of uterine tissues obtained from the middle of the uterine horn of a 2-year-old nulliparous Beagle (A) and an 8-year-old multiparous Beagle (B). Notice the myometrium of the nulliparous Beagle contains a thin stratum vasculare with only a few blood vessels (arrowheads), whereas the myometrium of the multiparous Beagle contains a thick stratum vasculare with numerous blood vessels. Groups of mainly circularly orientated additional smooth muscle fibers (arrows) are located between the stratum vasculare and perimetrium. Gomori trichrome stain; bar = 500 μm.



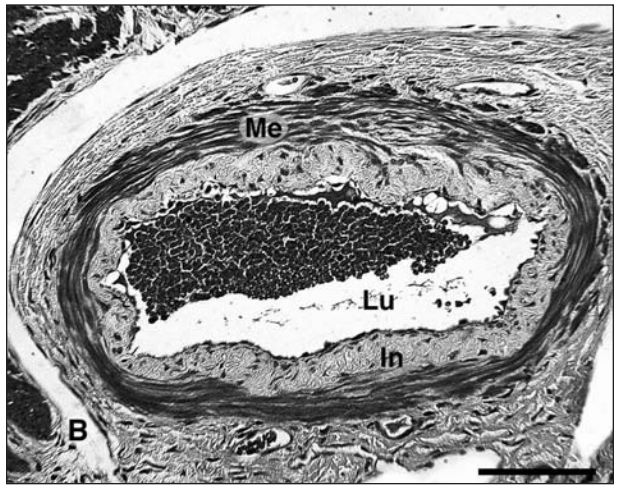


Figure 3—Photomicrographs of a cross-sectional view of the myometrial arteries in the uterus of a 2-year-old nulliparous Beagle (A) and an 8-year-old multiparous Beagle (B). Notice the extremely thin tunica intima (In) and thick, compact tunica media (Me) in the nulliparous Beagle and the thick tunica intima and thin tunica media in the multiparous Beagle. Lu = Lumen of the artery. Gomori trichrome stain; bar = 100 μm.

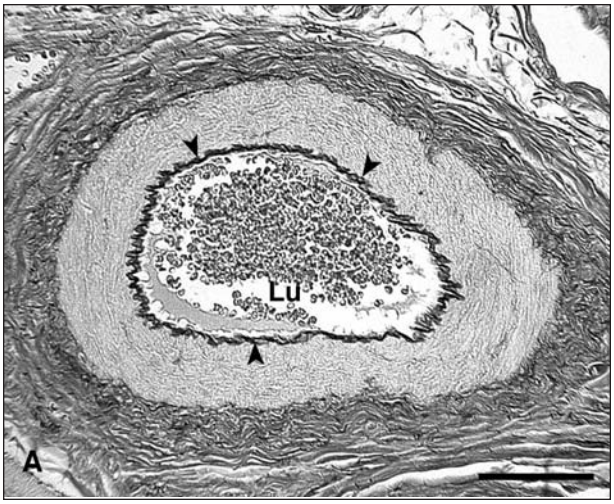
In contrast to uterine cross sections of the multiparous group, uterine cross sections of the nulliparous dogs had a bulge in the antimesometrial wall of the uterus and several superficial indentations in the remainder of the uterine wall.

The myometrium consisted of inner circular and outer longitudinal muscle layers. These layers were of similar thickness and enclosed the stratum vasculare (**Fig 2**). The essentially circularly oriented smooth muscle fibers of the inner muscle layer can form flat spiral coils, as suggested by examination of serial sections. The longitudinal muscle layer formed the base of the bulge in the antimesometrial wall observed in the nulliparous dogs (Fig 1). In the multiparous group, circularly orientated groups of additional muscle fibers were evident between the longitudinal muscle layer and the perimetrium. The perimetrium in the uteri of multiparous dogs was thicker than that of nulliparous dogs. Furthermore, the connective tissue of the perimetrium and myometrium contained slightly more elastic fibers in the multiparous group.

The stratum vasculare contained numerous sections through large blood vessels, whereas only a few small vessels were detected in the adjacent muscle layers. Typically, blood vessels of the stratum vasculare had greater external diameters and were more numerous in the multiparous dogs than in the nulliparous dogs (Fig 2). In the uteri of nulliparous dogs, the arterial sections were mainly round to oval. In the uteri of multiparous dogs, irregular round arteries predominated and frequent tangential sections of arteries were encountered.

Distinct differences in structures of the arterial wall were evident between groups. The arterial wall of each group consisted of the tunica intima, tunica media, and tunica adventitia. The tunica intima was composed of the endothelium and subendothelial lamina, which contained the internal elastic membrane. The tunica adventitia was not clearly discernible from the surrounding connective tissue.

Arteries of the uteri of nulliparous dogs were char-



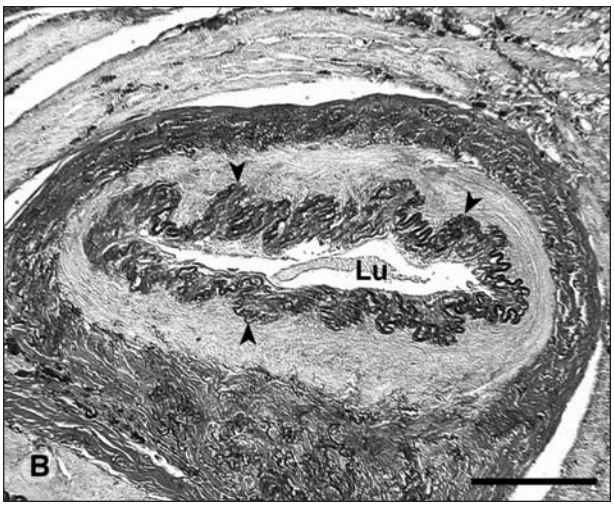
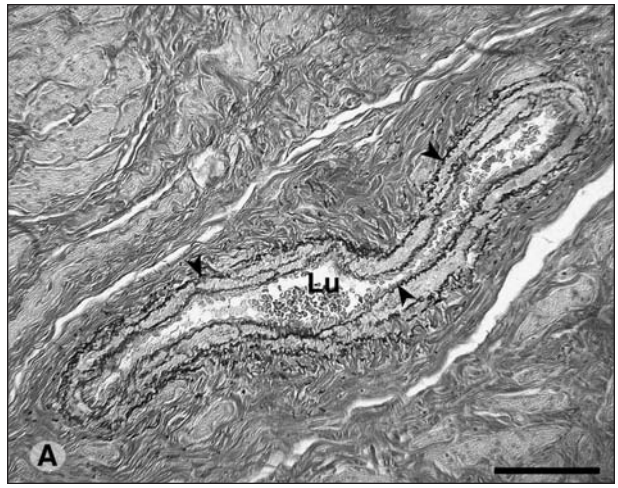


Figure 4—Photomicrographs of another cross-sectional view of the myometrial arteries in the uterus of a 2-year-old nulliparous Beagle (A) and an 8-year-old multiparous Beagle (B). Notice the myometrial artery of the nulliparous Beagle has a thin, homogenous, and wavy internal elastic membrane (arrowheads), whereas the myometrial artery of the multiparous Beagle has an internal elastic membrane that appears to be disintegrating, wide, and highly folded. Gomori trichrome stain; bar = 100 µm.



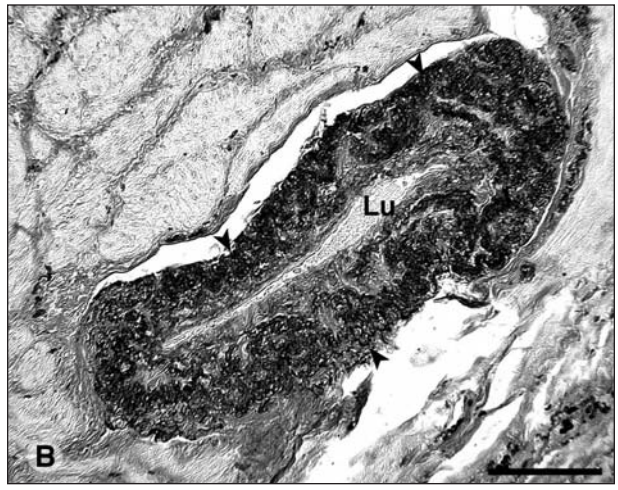


Figure 5—Photomicrographs of a cross-sectional view of the myometrial veins in the uterus of a 2-year-old nulliparous Beagle (A) and an 8-year-old multiparous Beagle (B). Notice there are few elastic fibers (arrowheads) in the wall of the myometrial vein of the nulliparous Beagle, whereas the wall of the myometrial vein of the multiparous Beagle has a large number of elastic fibers. Orcein stain; bar = 100 µm.

acterized by an extremely thin tunica intima and a thick compact tunica media of smooth muscle (**Fig 3**). The internal elastic membrane appeared as a relatively thin and homogeneous wavy line (**Fig 4**). In contrast, the tunica intima of the arteries in the uteri of multiparous dogs appeared thickened and the tunica media appeared to be thin. In addition, the intima bulged into the arterial lumen in places, and the internal elastic membrane appeared to be disintegrating and wide as well as highly and irregularly folded. Elastic fibers in the adventitial connective tissue of the arteries appeared to be more numerous in the multiparous group.

The wall of myometrial veins in multiparous dogs contained large numbers of elastic fibers. In contrast, distinctly fewer fibers were observed in the wall of corresponding vessels in nulliparous dogs (**Fig 5**).

The endometrium was bound by a cuboidal to columnar epithelium lining the lumen of the uterus and contained numerous secretory units of the uterine glands embedded in the propria connective tissue. The secretory units were also lined by cuboidal to columnar epithelium.

Morphometric analysis—All morphometric data had a normal distribution. Pertinent means of the compiled estimated variables from the various uterine tissue components were determined (**Table 1 and 2**). Mean volume fractions were also calculated (**Fig 6**).

Mean absolute volume of the uterine wall did not differ substantially between groups (Table 1). However, values for each dog were highly variable, ranging between 147.9 and 524.1 mm^3 in the nulliparous group and between 147.3 and 409.8 mm^3 in the multiparous group. In general, this variability was consistent with the considerable variation among dogs in the absolute volumes for other variables. Typically, the myometrium constituted approximately 75% and the endometrium 25% of the entire wall of the uterus in both groups (Table 2).

Estimated mean absolute volume of the myometrium in the nulliparous group was 234.5 mm^3 and con-

Table 1—Mean ± SEM estimated absolute volumes of the uterine wall, myometrium and endometrium and volume fractions of various uterine components in samples obtained from 7 nulliparous and 5 multiparous Beagles

Group	Uterine wall	Myometrium (mm^3)				Endometrium (mm^3)		
		Total	Muscle	Connective tissue	Blood vessels	Total	Glands	Remaining endometrium
Nulliparous	314.4 ± 51.0	**234.5 ± 40.3**	129.7 ± 17.2	96.0 ± 22.7	8.8 ± 1.1*	**79.9 ± 11.3**	34.1 ± 12.7	45.8 ± 6.6
Multiparous	308.2 ± 48.1	**229.7 ± 32.5**	137.0 ± 23.7	58.0 ± 10.7	34.6 ± 6.0*	**81.6 ± 19.6**	13.2 ± 3.0	65.3 ± 16.4

Values reported are number of cubic millimeters.
*Within a column, values differ significantly ($P = 0.005$) between groups.

Table 2—Mean ± SEM estimated relative volumes of the myometrium and endometrium and their tissue components in tissues obtained from 7 nulliparous and 5 multiparous Beagles

Group	Myometrium (%)				Endometrium (%)		
	Total	Muscle	Connective tissue	Blood vessels	Total	Glands	Remaining endometrium
Nulliparous	**74.2 ± 1.8**	57.5 ± 3.3	38.3 ± 3.4*	4.2 ± 0.7†	**25.8 ± 1.8**	36.8 ± 9.4	63.3 ± 9.4
Multiparous	**75.2 ± 3.2**	58.7 ± 4.7	25.0 ± 2.1*	16.3 ± 3.4†	**24.8 ± 3.2**	17.5 ± 2.7	82.5 ± 2.7

Values reported are percentages.
,†Within a column, values differ significantly ($P < 0.05$; †$P = 0.005$) between groups.

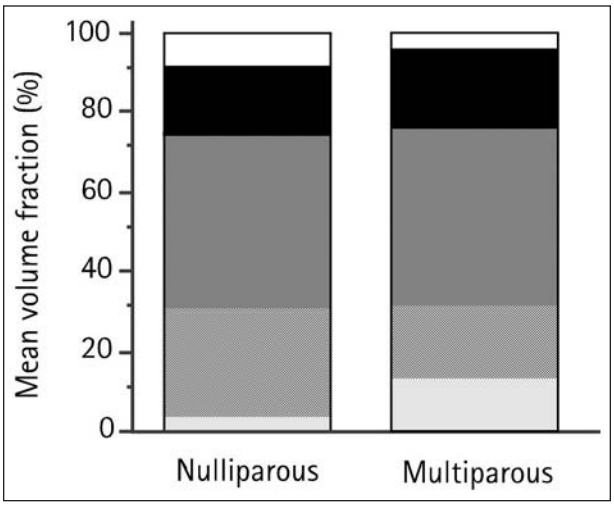


Figure 6—Stacked bar graph depicting mean volume fractions of various uterine tissue components for 7 nulliparous and 5 multiparous Beagles. Values for myometrial connective tissue and myometrial blood vessels differed significantly ($P < 0.05$) between groups. Components were as follows: endometrial glands (white), rest of endometrium (black), myometrial muscle (gray), myometrial connective tissue (diagonal stripes), myometrial blood vessels (cross-hatched).

sisted of 129.7-mm^3 (57.5%) smooth muscle, 96.0-mm^3 (38.3%) connective tissue, and 8.8-mm^3 (4.2%) blood vessels (Table 1 and 2). When comparing these values with values for the multiparous group, the absolute and relative volumes of the blood vessels (34.6 mm^3 [16.3%]) were significantly ($P = 0.005$ and $P = 0.003$, respectively) higher and the relative volume of the connective tissue (25.0%) was significantly lower. In both the nulliparous and multiparous groups, uterine arteries accounted for a larger portion of the blood vessel volume, compared with the portion for the veins.

Mean ratio of arterial wall thickness to external diameter was 0.254 in the nulliparous group and 0.278 in the multiparous group. These values differed significantly and revealed an increase in arterial wall thickness in the uteri of multiparous dogs.

Connective tissue (remaining endometrium) was the most prominent endometrial component and constituted 63.3% in the nulliparous dogs and 82.5% in the multiparous dogs (Table 2). Secretory units of the uterine glands accounted for 36.8% and 17.5% of the total endometrial volume in the nulliparous and multiparous dogs, respectively. Despite the relatively large differences of the mean absolute and relative volumes of these endometrial components between groups, the values did not differ significantly.

Analysis of results of the factorial ANOVA with the hormone concentrations and age of the dogs as covariants revealed that these cofactors did not influence results for evaluation of the morphometric results.

Discussion

The 2 homogenous groups of dogs used in the study reported here provided the basis for a meaningful morphometric comparison between the uteri of nulliparous and multiparous dogs. However, even the macroscopic examination revealed considerable variability among dogs with regard to size of the uterus. It is interesting that the greatest volume of the uterine wall was found in a nulliparous dog and the smallest volume was obtained from a multiparous dog. Cross sections from the cranial and caudal parts of the uterine horn did not differ from those obtained from the middle of the uterine horn, implying that the morphometric results obtained from the uterine specimens were representative of the entire uterine horn.

Several studies[3,a,j-l] have examined wall thickness, size, and weight of uteri in nulliparous and multiparous animals in other species; however, we are aware of only 1 study[b] that examined uteri in dogs. Those investigators concluded that pregnancy leads to an increase in wall thickness or size of the uterus. One

study[b] found an increase in thickness of all layers of the uterine wall in a single multiparous dog, compared with the thickness for 2 nulliparous dogs. This contrasts with results of the study reported here in which we did not detect significant differences in the volume of the uterine wall between nulliparous and multiparous dogs. These conflicting results are readily explained by the differing methods and number of dogs used. That other study[b] was based on measurements of uterine wall thickness in only 2 nulliparous dogs and 1 multiparous dog. Those investigators did not account for age, breed, and stage of the reproductive cycle. Considering the high variability of uterine wall volume among nulliparous and multiparous dogs in our study, comparison of only 1 or 2 dogs in each group could lead to incorrect conclusions.

As indicated previously, gravid uteri in horses, cattle, and swine do not revert to their initial size after involution.[3,a,j-l] This contrasts with the finding in our study of uteri in dogs. The discrepancy could be ascribed to the comparatively long involution time (at least 12 weeks) for the canine uterus.[12]

According to 1 study,[21] the canine uterus undergoes a permanent increase in size during the first estrous cycle, whereas subsequent estrous cycles do not influence the size of the uterus. On the basis of conclusions for that study and the study reported here, it can be deduced that the definitive size of the nongravid canine uterus is achieved after the first estrous cycle and that subsequent estrous cycles or possible pregnancies will not alter the size of the nongravid uterus.

The most distinct histomorphologic difference between the uteri of nulliparous and multiparous dogs was seen in the stratum vasculare and its arteries. The greater number of arterial cross sections and tangential sections in the uteri of multiparous dogs, compared with the number in the uteri of nulliparous dogs, implies coiling and possibly an increase in branching of the arteries in the uteri of multiparous dogs. The greatly increased thickness of the tunica intima and concomitant thinning of the muscular media in uterine arteries of multiparous dogs is referred to as pregnancy sclerosis and has been reported in other species.[3,22,23,a,j-l] In trichrome-stained sections, the thickened intima appeared to have the same shade of green as collagenous fibers of connective tissue. This suggested deposition of collagenous or other extracellular matrix material. The apparent loss of smooth muscle cells in the tunica media of the arteries was obviously more than compensated for by the thickened tunica intima. This led to a significant increase in arterial wall thickness in the multiparous dogs, compared with wall thickness in the nulliparous dogs.

On the basis of studies[22,24] of uteri in other species, arterial pregnancy sclerosis appears to be a cyclic phenomenon. In cows[24] and guinea pigs,[22] these alterations in the structure of arterial walls in the uteri of multiparous animals disappeared during pregnancy but were reestablished during subsequent involution. It could not be elucidated whether this is also the case in dogs. Interestingly, we also detected arterial pregnancy sclerosis in the uterus of a uniparous dog that we examined. Thus, the appearance of this feature in a histologic section of the canine uterus is proof of a previous pregnancy.

The distinct increase in the number of arterial sections and their relevantly thickened walls is consistent with the significantly higher blood vessel volumes in the uteri of multiparous dogs, compared with values for nulliparous dogs. Because we did not detect relevant differences in the absolute volume of the uterine wall between groups, we deduced that the significant increase in blood vessel volume in the uteri of multiparous dogs was compensated for by a decrease in the amount of myometrial connective tissue.

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[e] AnalySIS, Soft Imaging System GmbH, Münster, Germany.

[f] Wild Leitz Aristoplan, Leica Microsystems, Glattbrugg, Switzerland.

[g] Colorview 12, Soft Imaging System Corp, Lakewood, Colo.

[h] Philips Brilliance 180P, Philips Consumer Electronics Corp, Atlanta, Ga.

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