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Spontaneous ovulation in cats—Uterine findings and correlations with animal weight and age

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ABSTRACT

Cats are considered induced ovulating animals but ovulations occur in the absence of mating (i.e., spontaneous ovulations). Factors that stimulate such ovulations remain largely unknown. In this study, ovaries and uterine horn segments from 89 post-pubertal queens presented for ovariectomy were evaluated morphologically and histologically. It was hypothesized that corpora lutea (CL) are present in non-pregnant cats and can be associated with cystic endometrial hyperplasia (CEH). Cats were assigned to three age groups (7–12 months, $n = 32$; 13–24 months, $n = 26$ and ≥ 24 months, $n = 31$) and three weight groups (2.1–2.8 kg, $n = 28$; > 2.8–3.3 kg, $n = 32$ and > 3.3 kg, $n = 29$). Uterine horn diameter and thickness of the endometrium and myometrium were determined. Corpora lutea were detected in 39.3% of the cats and presence did not differ between age groups. The percentage of queens with CL increased with bodyweight (2.1–2.8 kg: 14.3%; > 2.8–3.3 kg: 37.5%, > 3.3 kg: 65.5%; $P < 0.01$). In cats with CL, the thickness of all layers of the uterus were greater than in cats without CL ($P < 0.05$). Of the cats, 22.5% had CEH but there was no difference between cats without and with CL. The percentage of CEH increased with age ($P < 0.001$) but did not differ between weight groups. In conclusion, ovulations occurred in the absence of mating in approximately one third of all queens.

1. Introduction

Cats are often considered strictly to be animals where there is only mating-induced ovulations (Concannon et al., 1980; Wildt et al., 1980; Robison and Sawyer, 1987) but spontaneous ovulations (ovulation in the absence of mating) have been reported (Gudermuth et al., 1997; Lawler et al., 1993; Pelican et al., 2005). Corpus luteum (CL) formation in cats not induced by mating to have ovulations appears to be more frequent than assumed in veterinary textbooks (Arthur et al., 1996; Ortega-Pacheco et al., 2012). Over a 3-month observation period, 10 of 15 colony-housed queens had between one and three ovulations when there was no mating. The presence of a tomcat kept in the same room but in a separate cage further increased the number of spontaneous ovulations and subsequently there was CL development in these queens (Gudermuth et al., 1997). In another study, 56% of queens observed during a 3-month period had at least one ovulation not associated with mating (Pelican et al., 2005).

Factors that stimulate or predispose queens to have spontaneous ovulations or CL formation remain largely unknown or are at least speculative. Tactile contact with other females in cat colonies, stroking of the cats´ back and neck by humans and stress of

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experimental procedures have been suggested as factors that can induce spontaneous ovulations in cats (Arthur et al., 1996), but there is no experimental or clinical evidence to support any of these possibilities. Spontaneous ovulations occur also in queens caged individually without tactile contact with humans even when these queens are maintained in a stress-free environment. Thus, seven out of 20 individually caged queens had ovulations once or repeatedly over a 9-month observation period (Lawler et al., 1993). Considering six of these cats that had ovulations were at least 7 years old, an increase in spontaneous ovulations with age was suggested (Lawler et al., 1993), but results from comprehensive studies on ovulations in queens of different ages to the best of our knowledge have not been reported.

The duration of CL functions in non-pregnant queens lasts between 40 and 45 days (Paape et al., 1975) whereas CL in pregnant cats persist for 60–63 days (Verstegen et al., 1993). Based on luteal production of steroidogenic enzymes and on steroid contents of the CL, the steroidogenic capacities of CL at the same stage do not differ between pregnant and non-pregnant domestic queens and felids in their natural habitat during luteal phases (Jewgenow et al., 2012; Zschockelt et al., 2014).

Similar to the pathogenesis of canine pyometra, prolonged actions on the endometrium of progesterone in cats have been suggested as a major predisposing factor for feline pyometra. In cats with cystic endometrial hyperplasia or pyometra, CL are more frequent than in non-pregnant cats without CL (Lawler et al., 1991, 1993; Potter et al., 1991). Spontaneous ovulations and subsequent physiological changes as a consequence of CL hormonal secretions may thus contribute in the onset of feline pyometra (Lawler et al., 1993; Swanson, 2019).

In the present study, ovaries and distal uterine horn segments were collected from queens presented for routine ovariectomy. Ovaries and uterine horns were evaluated for morphological appearance and histological structures. It was hypothesized that frequency of presence of CL in non-pregnant cats is positively correlated with age of the cats and is associated with the incidence of endometrial hyperplasia.

2. Materials and methods

2.1. Animals

Ovaries and distal uterine horn segments were obtained from 106 cats which were mostly postpubertal. The cats were clinically healthy queens presented for routine ovariectomy at the Vetmeduni Vienna Division of Obstetrics and Reproduction between July 2017 and January 2018. The reasons to perform an ovariectomy were not related to the study and thus no animal experimentation license was required. Prior consent of the owners for scientific use of the material removed at the time of surgery was obtained.

All cats were adopted by their owners from the same animal shelter between 1 and 355 days (96 ± 74 days, mean ± standard deviation [SD]) before surgery. Legislation in Austria requires all queens with free outdoor access and not explicitly intended for breeding to be spayed; spaying was offered to the new cat owners by Vetmeduni Vienna in cooperation with the animal shelter. In total, cats were clinically examined at least four times before spaying. None of the queens had symptoms of general or gynecological disorders and no cat was pregnant when ultrasonography of the uterus was conducted. The shelter allocated uncastrated female cats only to single-cat households (n = 50; 47%) or together with other queens or castrated tomcats (n = 56; 53%) but not gonad-intact tomcats. According to the owners, queens had not been in contact with gonad-intact males. In none of the cats were there symptoms of pregnancy detected during the study.

Ten of the cats were born at the animal shelter while others had a previous owner who could provide information about their age. The remaining cats were found and transferred to the shelter. In these cases, age was estimated based on their dentition and, in older animals, also on their general condition and fur texture. Cats were estimated to be 2 to 4 weeks old, when their deciduous incisors became visible, 3 to 4 weeks old after eruption of the canines and 4 to 6 weeks after eruption of the premolar teeth. Thereafter, the age was estimated based on the dentition changes (4, 5 and 6 months, respectively, when the incisors, canines and premolars were changed). Molars should be visible between 5 and 8 months. Furthermore, the extent of discoloration and the general state of the permanent teeth were assessed. Slight yellowish discoloration begins at about 2 years and intensifies up to the age of 5 years. In queens that were classified to be 5 or more years old, the state of the teeth was included in the evaluation. Age of the queens used in this study ranged from 4 months to 9 years (21.6 ± 19.8 months, mean ± SD)

Animals were weighed before induction of anesthesia and bodyweight ranged from 1.9 to 4.3 kg (3.1 ± 0.5 kg, mean ± SD). The queens were of different breeds but 88% (n = 93, 87.7%) were European shorthair cats. To obtain data from a homogeneous group of animals, only data from European shorthair cats older than 6 months were included in the analysis. Thus, data from 89 of the 106 total cats were used for the present study.

For statistical comparisons, cats were assigned by age to three groups: 7–12 months (n = 32, 9.0 ± 1.7 months [mean ± SD]), 13 to 24 months (n = 26; 15.8 ± 3.1 months) and > 24 months (n = 31, 43.4 ± 17.3 months, range 25–99 months). Cats were also divided by weight into three groups of approximately equal size: ≤ 2.8 kg (n = 28, 2.6 ± 0.2 kg, range 2.1–2.8 kg), > 2.8–3.3 kg (n = 32, 3.1 ± 0.2 kg) and > 3.3 kg (n = 29, 3.1 ± 0.5 kg, range 3.3–4.3 kg). Age groups did not differ in weight (P = 0.13) nor weight groups by age (P = 0.15) but there was a slight correlation between values for age and weight (r = 0.246, P = 0.02).

2.2. Surgical procedures

Before surgery, all cats were classified as normal healthy animals according to the ASA (American Society of Anesthesiologists) classification system (Saklad, 1941; Clarke and Hall, 1990). Anesthesia was induced with 30 µg/kg medetomidine (Narcostart 1 mg/ml, Le Vet B.V., Oudewater, The Netherlands) in combination with 0.2 mg/kg butorphanol (Alvegesic vet 10 mg/ml, Alvetra und

Werfft, Vienna, Austria) and 5 mg/kg ketamine (Ketamidor 100 mg/ml, Richter Pharma, Wels, Austria). If necessary, anesthesia was maintained with isoflurane in oxygen. All cats received meloxicam (0.1 mg/kg intraoperative followed by 0.05 mg/kg orally for 3 days once daily; Metacam, Boehringer Ingelheim, Ingelheim, Germany). Routine ovariectomy with partial removal of the uterine horns was performed via a small ventral midline incision. In case of obvious pathological abnormalities, the uterus was removed completely. The abdominal wall was closed in three layers with uncoated monofilament suture material (Biosyn 3/0, Covidien, Vienna, Austria).

2.3. Histology and histopathology

Immediately after surgery, the ovaries and uterine horn segments were examined macroscopically and abnormalities were noted. Ovaries and uterine horn segments were then transferred into 4% neutral buffered formaldehyde, where these tissues were stored for no longer than 48 h at room temperature. Ovaries were cut longitudinally and uterine horn segments transversally 1 cm caudal to the horn tip and after dehydration in ethanol these tissues were embedded in paraffin wax. Sections (3 µm thick) were cut from the paraffin tissue blocks using a microtome and were mounted on glutaraldehyde coated–slides, dried overnight at 37 °C and were stained with haematoxylin-eosin for histologic evaluation.

For histological examinations, slides were assessed using the Aperia CS2 image capture device (Leica Biosystems, Nussloch, Germany). Sections were cut to size using an Adobe Photoshop raster graphics editor (Adobe Systems, San José, California, USA). Measurements were conducted using the Icy open community platform for bioimage informatics (BioImageAnalysis.org, Institute Pasteur, Paris, France) and the following variables were evaluated: uterine horn diameter (mean of two lines orthogonal to each other), thickness of the endometrium (luminal epithelium and Lamina propria mucosae), internal (circular) layer width of the myometrium, and middle (vascular) plus external (longitudinal) layer width of the myometrium plus perimetrium. For further analysis, the thickness of all three layers of the myometrium was combined. All measurements were repeated at four different locations.

With histological examinations, there were assessments for characteristics of CEH such as proliferation and secretion of the endometrial glands and consequently induced cystic distension. Diameter of the endometrial glands was measured (mean of two lines orthogonal to each other). The severity of CEH was defined as mild with a minimum of four dilated glands per microscopic visual field (200 x magnification) with a diameter of greater than 100 µm. Moderate CEH was defined as there being at least four dilated glands of more than 400 µm in diameter and there was the classification of severe CEH when there were four dilated endometrial glands that were more than 800 µm in diameter (Fig. 1A–D).

2.4. Statistical analysis

Statistical analyses were performed using the IBM SPSS Statistics software (version 25.0; Armonk, NY, USA). Data were assessed for normal distribution using the Kolmogorov-Smirnov test and for homogeneity of variances using the Levene test. Because there was neither a normal distribution of data nor a homogeneity of variances for all values of variables, non-parametric tests were used for all

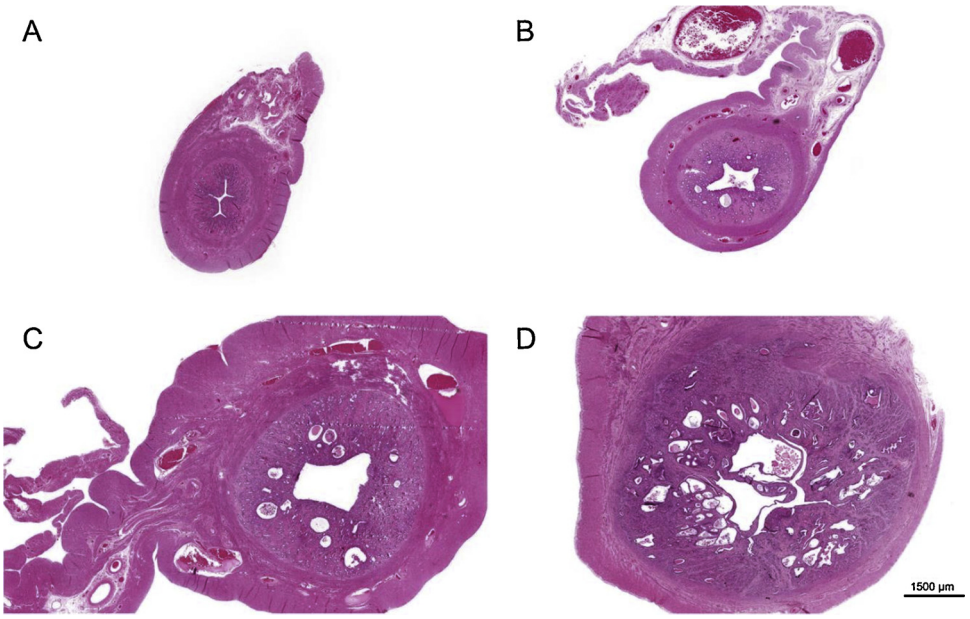

Fig. 1. Examples of uterine horn segments with (A) no, (B) mild, (C) moderate and (D) severe cystic endometrial hyperplasia (HE staining/Bar = 1500 µm).

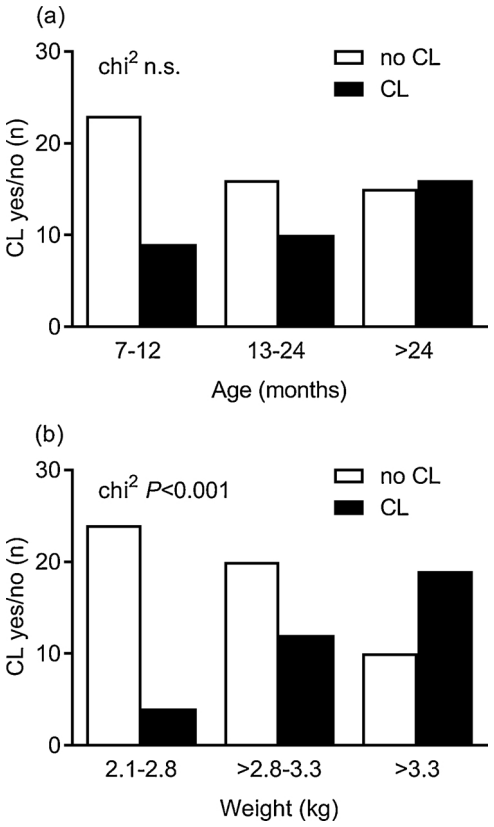

Fig. 2. Presence of corpora lutea as a result of spontaneous ovulations (ovulations in the absence of mating) in cats (n = 89) of (a) different age groups and (b) different weight groups, differences among groups are indicated in the figures.

statistical analyses. For none of the variables were there differences between the left and right uterine horn segment and ovary, respectively. For all statistical comparisons measurements from the left and right side were, therefore, combined and averaged. Groups were compared using non-parametric tests for independent samples (Kruskal-Wallis-H-test and Mann-Whitney-*U*-test for three and two groups, respectively) and frequency distributions were analyzed using the Chi square-test. Measurements obtained from the left and right ovary and uterine horn segment in the same animals were compared using the Wilcoxon test. The Spearman´s coefficient of correlation was used to determine correlations between weight and age of the queens. Data are reported as the mean ± SEM. *P*-values of < 0.05 were considered significant.

3. Results

There were CL in 39.3% of the cats (n = 35). The number of CL per animal (i.e., in both ovaries) ranged from one (three cats) to ten (one cat) with a mean of 3.9 ± 1.9 CL in all cats with at least one CL. The frequency in presence of CL did not differ among age groups. Among 7 to 12 months-old queens (n = 32), 28.1% had a CL while in queens from 13 to 24 months (n = 26) and queens older than 24 months (n = 31), the percentage of animals with CL was 38.5% and 51.6%, respectively (Figs. 2a and 3).

There were obvious differences in frequency of CL presence with there being an increased frequency of CL as body weights of queens increased. In the group of queens weighing 2.1–2.8 kg (n = 24), between 2.8 and 3.3 kg (n = 32), and more than 3.3 kg (n = 29), there were CL in 14.3%, 37.5%, and 65.5%, respectively ($P < 0.01$ among groups; Fig. 2b). The frequency of CL presence was greater ($P < 0.05$) in queens housed individually (23/46, 50%) than in queens housed together with other female or castrated male cats (12/43, 28%).

In cats with CL, the diameter of the uterus and the thickness of the endometrium and the myometrium were greater than in cats without CL, independent of age and weight (e.g., uterine diameter in cats with CL 5.8 ± 1.4 mm, cats without CL 4.8 ± 3.2 mm, $P < 0.001$, Fig. 4). Diameter of the uterus and thickness of the endometrium and myometrium increased with age ($P < 0.05$; Fig. 5a) and with weight of the queens ($P < 0.05$ Fig. 5b).

Based on histological findings, 22.5% of cats were classified as having cystic endometrial hyperplasia (CEH) but there was no difference between cats without CL (22.2% CEH) and with CL (22.9% CEH). The frequency of CEH increased with age ($P < 0.001$; Fig. 6a) but did not differ among weight groups (Fig. 6b).

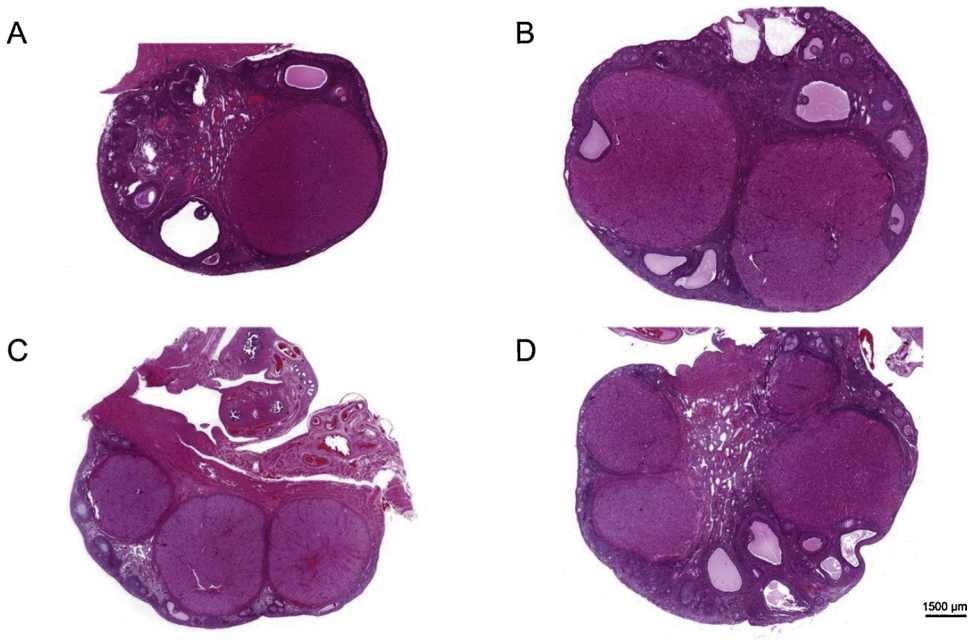

Fig. 3. Representative ovaries with (A) single and (B–D) multiple corpora lutea detectable with histological examination (HE staining, Bar = 1500 µm).

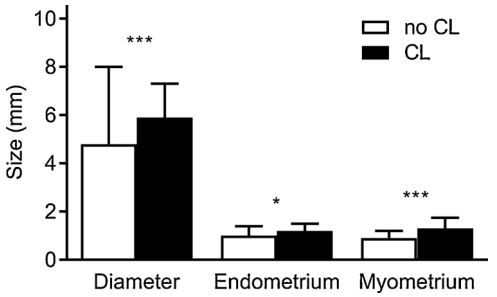

Fig. 4. Uterine diameter and thickness of the endometrium and myometrium in cats with (n = 35) and without corpora lutea (CL; n = 54), differences between groups are indicated in the figure (*P < 0.05, ***P < 0.001).

4. Discussion

The results of this study indicate spontaneous, non-mating induced ovulations occur in approximately one third of all queens. Considering CL regress after 40–45 days in non-pregnant cats (Paape et al., 1975), the percentage of cats with spontaneous ovulations during a longer observation period (Lawler et al., 1993) can be assumed to be even greater.

Results from the present study do not allow a strict differentiation between CL formation by ovulation or luteinization of follicles from which there was not an ovulation. Remains of an oocyte or zona pellucida indicative of non-ovulatory CL formation were not detected in any of the ovaries, but because there was assessment of only one sectional plane per ovary, the possibility of luteinization of follicles from which there was not ovulation cannot be excluded. Based on the histological appearance of the CL and consistent with results from previous studies when there was either ovaries obtained at the time of ovariectomies or based on progesterone profiles (Lawler et al., 1993; Gudermuth et al., 1997; Pelican et al., 2005), the term ovulation is used throughout this manuscript. With the cat being a polyovulatory species, most queens have multiple CL, and there was a single corpus luteum in only three cats in the present study. Even in these three animals, additional CL could have been present but not detected using the procedures utilized in the present study.

In the present study, as a consequence of the use of only privately owned cats, there are some limitations compared with studies with more homogenously managed experimental animals. Queens were maintained as indoor cats in typical private households and thus in similar housing conditions but not as homogenous as would have been the case if cats were housed in more controlled laboratory settings. All queens, however, were adopted by their owners from the same animal shelter where they had been clinically evaluated routinely for a minimum of 14 days by the professionals of the research group conducting the present study. In addition to at least two examinations at the animal shelter, queens were examined pre-operatively and at all times were clinically healthy and not

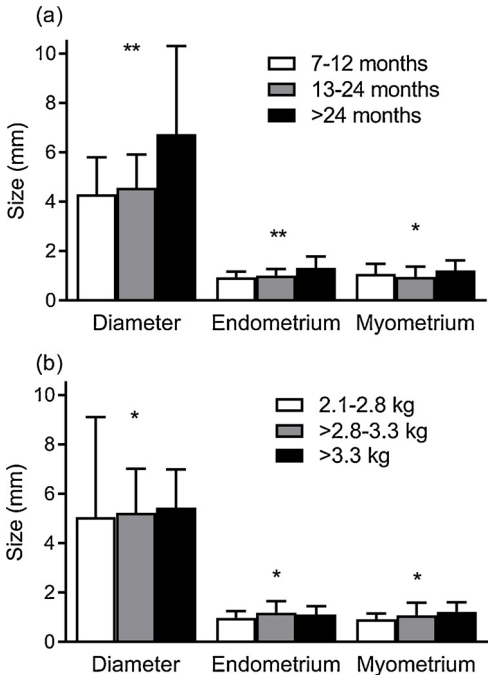

Fig. 5. Uterine diameter and thickness of the endometrium and myometrium in cats (n = 89) of (a) different age groups and (b) different weight groups, differences among groups are indicated in the figures (*P < 0.05, **P < 0.01).

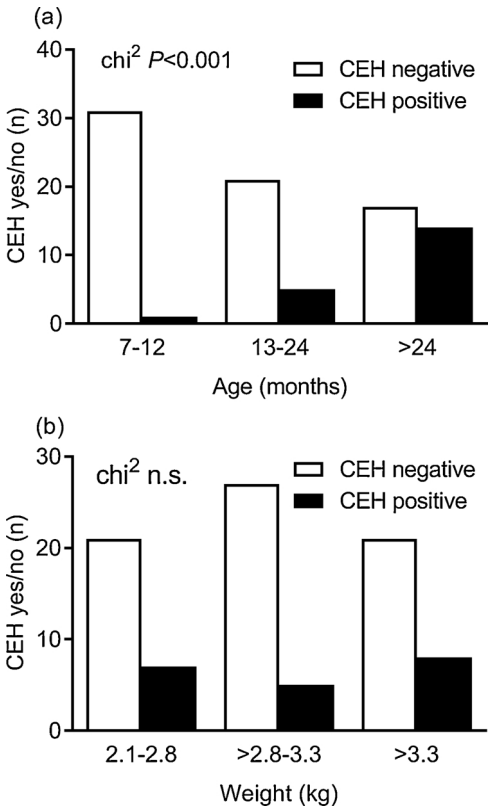

Fig. 6. Diagnosis of cystic endometrial hyperplasia in cats (n = 89) of (a) different age groups and (b) different weight groups, differences among groups are indicated in the figures.

detected to be pregnant. Based on input from the owners, none of the cats had been in contact with intact males during the period they were in the owner's possession prior to the present study. In individual cases, however, owner information may have been incorrect and thus it is not possible to exclude, with certainty, that mating had not occurred. Because it is unlikely that there was a mating with a non-fertile tomcat, the number of mated queens, if any, would be few and, therefore, there should be little to no data bias as a result of mating induced ovulations during the present study. Researchers are legally obliged considering the present governmental regulations to reduce and replace animal experimentation in their experimental endeavors (e.g., Smith, 2001). Experimental surgeries, therefore, may not be justifiable when similar results can be obtained without animal experimentation such as what occurred in the present study. These considerations were taken into account when designing and conducting the present study.

The percentage of cats with CL increased with weight of the animals and in the heaviest group approximately two thirds of the queens had CL. Body weight groups did not differ as a result of age and there was only a slight correlation between values for body weight and age of queens. It, therefore, is suggested that there is an increased frequency of CL that is associated with a greater body weight of queen cats.

Nutritional energy supply and the amount of body adipose tissue are key modulators of cyclic ovarian functions in many species and different physiological situations. In boars located in their natural habitat, body weight and reproductive functions are positively correlated and when food is scarce the percentage of estrous cyclic females and litter size are less (Massei et al., 1996). In women, there is an association with suckling duration during lactation and inhibition of ovarian follicular development as well as pituitary and gonadal hormone secretion. This negative effect is no longer present when there is child weaning from breast feeding. Lactational amenorrhea is most evident in poorly nourished traditional populations in developing countries and is markedly shorter in duration in well-nourished women (Tracer, 1996; McNeilly, 2001; Domer et al., 2015). The effect of energy stores in the body on ovarian functions is mediated in part by leptin, a hormone synthesized and released from adipose tissue. Leptin can modulate ovarian steroidogenesis directly and acutely in ruminants and is part of the regulatory system whereby nutritional status modulates reproductive functions (Kendall et al., 2004). The results from the present study indicate that a certain extent of over-nutrition in cats may enhance the occurrence of spontaneous ovulations and a potential involvement of leptin should be addressed in further studies.

Body condition score might have been a more reliable indicator of the nutritional status of queens in the present study, although weight can be more accurately determined. Because the initial hypothesis in the present study did not include nutritional status, body condition score was not assessed while weight was determined as part of pre-anesthesia procedures. To reduce an effect of non-nutrition-related weight differences, only cats of the same breed were included in the present study.

Tactile contact with other animals in cat colonies has been thought to induce ovulation in cats (Arthur et al., 1996) but frequent spontaneous ovulations have also been reported in experimental cats caged individually (Lawler et al., 1993). In the present study, a greater number of individually-housed than group-housed cats had CL. Results from the present study, therefore, do not support the hypothesis that interactions with other cats without mating induce ovulation in queens. Because the queens in the present study had been recently adopted by their owners, a certain extent of stress cannot be excluded. The stress associated with adaptation by a new owner might be more pronounced in queens especially when there are interactions with conspecifics that the queen had not previously interacted. Stress-induced anovulation exists in different animal species (e.g., sheep: Ehnert and Moberg, 1991; pigs: Einarsson et al., 2008; mice: Kala and Nivsarkar, 2016) as well as women (Berga et al., 1997) and may explain why ovulations were more frequent in the individually-housed cats in the present study.

Contrary to the initial hypothesis for the present study, frequency of CL did not increase with the age of queens. Based on a limited number of animals, an increase in spontaneous ovulations with age has been suggested previously (Lawler et al., 1993), however, results of the present study are inconsistent with these previous suppositions. To exclude prepubertal animals, only queens older than 6 months of age were included in the present study. Although many cats reach puberty at 4 months of age (Welsh et al., 2013), there may be exceptions where puberty occurs before this age. The exact age of the cats was only known in those animals born at the animal shelter and had to be estimated in most of the other animals. An adequate safety margin, therefore, was considered for inclusion of queens in the present study that had attained puberty before initiation of this study.

The presence of CL was associated with a larger uterine diameter and increased thickness of all layers of the uterine wall compared to animals without CL. The hypertrophy of uterine tissue during the luteal phase that was detected in the present study is consistent with findings in other species (e.g., sheep: Johnson et al., 1997; horses: McDowell et al., 1987; Ferreira-Dias et al., 2001). Progesterone stimulates uterine protein synthesis and induces the production of histotroph by the endometrial glands (Kenney, 1978; McDowell et al., 1987). Findings in queens of the present study are thus indicative of normal endocrine competence of CL. Irrespective of the presence or absence of CL, uterine diameter and thickness of the uterine wall increased with age and there was a lesser association of the values for these variables with body weight than there was with the age of queens.

Prolonged actions of progesterone on the endometrium in non-pregnant cats has been suggested to contribute to cystic endometrial hyperplasia and thus is a major predisposing factor for feline pyometra (Lawler et al., 1991, 1993; Potter et al., 1991; Swanson, 2019). Results from the present study, however, do not support this assumption. In contrast, the percentage of queens diagnosed histologically with CEH did not differ among animals without and with CL. Cystic endometrial hyperplasia was more often diagnosed with increasing age of the queens. To the best of our knowledge, there has been no previous research on the prevalence of CEH as related to the age of queens. Approximately 20% of the queens were diagnosed with CEH, which is similar to that in non-ovariectomized female dogs (Moxon et al., 2016). Similar to results with queens in the present study there is also in breeding bitches an increase in prevalence of CEH with increased age from 7% to 60%, in 2- compared with 6-year-old bitches, respectively (Moxon et al., 2016).

In bitches, CEH seems to be an abnormal response to the estrous cycle-dependent stimulation of endometrial gland proliferation by progesterone and estrogens. Characteristics of CEH are an increase in the number and size of endometrial glands, thickened

endometrium and increased secretory function (Barrau et al., 1975). With spontaneous ovulation in queens occurring more frequently than previously assumed, in the uterus of non-pregnant queens, there is a greater frequency than previously assumed of cyclic changes in progesterone and estrogen concentrations. As with bitches, this has to be assumed to be the underlying cause for the development of CEH in queens. Conception rate and litter size are reduced in bitches affected with CEH (England et al., 2012a,b). Results from the present study, therefore, are of clinical relevance to fertility of cats. Furthermore, the changes associated with CEH can result in conditions that predispose the queens to the development of pyometra (Bigliardi et al., 2004; De Bosschere et al., 2001). As for CEH in the present study, pyometra was more commonly diagnosed with increasing age in queens (Hagman et al., 2014) indicating CEH is related to uterine pathologies in bitches and queens. Studies of uterine lesions in cats that rely on presentation to a veterinarian when clinical signs are manifested may result in an underestimate of the actual proportion of queens affected by CEH because of the lack of overt symptoms in mild cases.

In conclusion, based on the results of the present study, spontaneous ovulations and CL development in cats is more frequent than previously assumed. The frequency of CL increased with weight of the queens, indicating there is a positive effect of body energy stores and proportion of adipose tissue on ovulation. The occurrence of CEH increased with age of the queens in the present study, indicating an increase in disruptions of endometrial proliferation with repeated cyclic changes in the pattern of estrogens and progesterone in the uterine tissues.

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Declaration of Competing Interest

The authors declare that they have no financial or personal relationship with other people or organisations that could inappropriately influence or bias this article.

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