

# Onset of reproductive capacity in unowned free-roaming cats

Ellie Bohrer,<sup>a</sup> Devany Billings,<sup>b</sup> Kristin Patton,<sup>c</sup> Michelle Kutzler<sup>b</sup>

<sup>a</sup>VCA North Portland Veterinary Hospital, Portland, Oregon, USA

<sup>b</sup>Department of Animal and Rangeland Sciences, Oregon State University, Corvallis, Oregon, USA

<sup>c</sup>Four Star Quarter Horses, Ostrander, Ohio, USA

## Abstract

Purpose was to investigate if an underlying biological cause exists for the reproductive success in unowned free-roaming (UFR) cats. These cats were presented for surgical sterilization during late summer/early fall. After castration of UFR toms, presence of penile spines was recorded, slides were made from vas deferens secretions for sperm morphology analyses, and testes were hemi-sectioned, formalin-fixed, paraffin-embedded, sectioned, stained with hematoxylin and eosin, and seminiferous tubule diameters were measured. After ovariectomy of UFR queens, total ovarian-uterine weights were recorded. Ovaries were hemi-sectioned, formalin-fixed, paraffin-embedded, sectioned, stained, and follicles were counted. Presence of penile spines did not differ ( $p = 0.07$ ) between toms 2 - 6 ( $n = 13$ ) and > 6 months ( $n = 16$ ). Percentage of normal sperm morphology did not differ ( $p = 0.39$ ) between toms 2 - 6 ( $n = 11$ ;  $77 \pm 11\%$ ) and > 6 months ( $n = 9$ ;  $81 \pm 13\%$ ). Seminiferous tubular diameter increased ( $p < 0.01$ ) in toms 2 - 2.5 ( $n = 6$ ;  $88.1 \pm 10.9 \mu\text{m}$ ), 3 - 4 ( $n = 6$ ;  $109.8 \pm 8.9 \mu\text{m}$ ), 5 - 6 ( $n = 6$ ;  $142.2 \pm 16.9 \mu\text{m}$ ), and > 6 months ( $n = 6$ ;  $237.9 \pm 52.5 \mu\text{m}$ ). There was no association ( $R^2 = 0.20$ ;  $p = 0.07$ ) between age and total ovarian-uterine weight of queens. Number of primary, secondary, and tertiary follicles recorded did not differ ( $p = 0.08$ ) between queens 2 - 4 ( $n = 13$ ) and 5 - 6 ( $n = 4$ ) months. The largest follicle diameter did not differ ( $p = 0.14$ ) between queens 2 - 4 ( $581.6 \pm 53.7 \mu\text{m}$ ) and 5 - 6 months ( $469.4 \pm 113.9 \mu\text{m}$ ). Results may explain why UFR cat populations continue to increase despite investments in extensive trap-neuter-return efforts. Selective pressure responses to shortened lifespans may contribute to onset of earlier reproductive capacity in UFR cats.

**Keywords:** Feral cats, fertility, puberty, reproduction

## Introduction

Unowned free-roaming (UFR) cats are cats that live within managed outdoor colonies. These cats are ecological threats to native habitats and their inhabitants. They are considered so invasive in nature that they have been listed among the hundred worst nonnative invasive species in the world.<sup>1</sup> A study<sup>2</sup> that analyzed 229 cases of feline impacts on islands revealed that UFR cats caused or contributed to 33 of the bird, mammal, and reptile extinctions. In the contiguous United States, 89% of the  $12.3 \times 10^9$  cat-caused mammal mortality is by UFR cats.<sup>3</sup> These cats also serve as vectors for numerous diseases, including feline panleukopenia virus, feline leukemia virus, feline immunodeficiency virus, and rabies. Additionally, *Toxoplasma gondii* (*T. gondii*) from UFR cats indirectly impacts marine ecosystems as *T. gondii* from cat feces leeches into marine environments through freshwater sources.<sup>4</sup> This has led to higher quantity of *T. gondii* in southern sea otters (*Enhydra lutris nereis*) and Hawaiian monk seals (*Monachus schauinslandi*).<sup>4</sup>

Problems associated with UFR cats are exacerbated by their sizable population. Current population estimate of UFR cats in the United States is somewhere between 30 and  $80 \times 10^6$  individuals.<sup>5</sup> Additionally, a sexually mature domestic female cat can produce up to 5 litters of kittens each year.<sup>5</sup> The most publicly supported sterilization program in the United States, Great Britain, Canada, the Netherlands, and Denmark is trap-neuter-return (TNR).<sup>6</sup> Even with the efforts placed into TNR programs, humane societies in the United States still cannot surgically sterilize UFR cats fast enough to control their numbers.<sup>7</sup> Additionally, these programs are expensive. Various estimates place neutering costs of UFR cats in TNR programs around  $\$70.77 \pm 11.75$  per cat nationally.<sup>8</sup> With the population of UFR cats in the United States alone, it would cost  $\sim \$4.2 \times 10^9$  to sterilize all individuals.

Since UFR cat populations continue to increase even with extensive TNR efforts, it is important to understand why UFR cats are so reproductively successful. A possible explanation

for their reproductive success is an earlier onset of reproductive capacity. Puberty in owned domestic cats occurs ~ 8 - 10 months in queens and 7 - 12 months in toms; however, this can vary depending on body condition, breed/body weight, and season.<sup>9,10</sup> We hypothesized that UFR cats gain reproductive capacity earlier than 6 months. Purpose was to analyze reproductive parameters of UFR toms and queens from 2 to 6 months of age.

## Materials and methods

### Tom study 1

Unowned free-roaming toms were presented for castration at a humane society in Oregon during August through October. Age was estimated by dental eruption using Silver's chart.<sup>11</sup> Age groups were as follows: 2 - 2.5 months (n = 6), 3 - 4 months (n = 6), 5 - 6 months (n = 6), and > 6 months (n = 6). After general anesthesia was induced, scrotal hair was clipped and the skin was aseptically prepared for surgery. A routine open castration was performed, and both testes from each cat were obtained. All toms recovered from surgery uneventfully. Tissues were hemi-sectioned, formalin-fixed, paraffin-embedded, cut into 6 µm sections, and stained with hematoxylin and eosin.

Seminiferous tubular diameter and presence of spermatogenesis within the seminiferous tubule lumen was determined at 200 x magnification using a bright field microscope with image capture capability. A single observer (EB) blinded to the tom's age measured perpendicular diameters of 5 randomly selected seminiferous tubules for each tom. In addition, the spermatogenesis score and Leydig cell density score for each tom was determined by a board-certified anatomic pathologist (KP) blinded to tom's age. A modified version of the Yoshida scoring method<sup>12</sup> was used to determine seminiferous tubular scores (Table 1). Leydig cell density within the interstitium was scored on a 0 - 3 scale: 0 = no cells present; 1 = scattered/few cells present; 2 = moderate number of cells present; and 3 = densely packed.

### Tom study 2

Unowned free-roaming toms were presented for castration at a feral cat neutering clinic in Corvallis, Oregon, during September. Age was determined by dental eruption using Silver's chart.<sup>11</sup> Age groups were as follows: 2 - 6 months (n = 13) and > 6 months (n = 16). After general anesthesia was induced, scrotal hair was clipped and the skin was aseptically prepared for surgery. Penis was examined to determine whether spines were present. A routine open castration was performed and vas deferens were used for further analyses. All toms recovered from surgery uneventfully. Fluid contents from both vas deferens were digitally expressed onto a prewarmed microscope slide and mixed with 1 drop of eosin-nigrosin morphology stain, spread with a spreader slide, and allowed to air dry. Sperm morphology analysis was performed under oil immersion (1000 x) using a bright field microscope with image capture capability. Fifty sperm were evaluated per slide by a board-certified theriogenologist (MK) blinded to tom's age, unless there were < 50 sperm present on the entire slide. These samples were not included in the sperm morphology evaluation. Sperm were categorized as having normal or abnormal morphology.

**Table 1.** Seminiferous tubular scores (modified from<sup>12</sup>)

Score	Criteria
12	Many late spermatids or sperm ( $\geq 10$ )
11	Only a few late spermatids or sperm ( $< 10$ )
10	No sperm or late spermatids, but many round spermatids ( $\geq 10$ )
9	No sperm or late spermatids, but only a few round spermatids ( $< 10$ )
8	No sperm or spermatids, but many secondary spermatocytes ( $\geq 10$ )
7	No sperm or spermatids, but only a few secondary spermatocytes ( $< 10$ )
6	No sperm, spermatids, or secondary spermatocytes, but many primary spermatocytes ( $\geq 10$ )
5	No sperm, spermatids, or secondary spermatocytes, but only a few primary spermatocytes ( $< 10$ )
4	No sperm, spermatids, or spermatocytes, but many spermatogonia ( $\geq 10$ )
3	Only germ cells present are a few spermatogonia ( $< 10$ )
2	Absence of germ cells, but Sertoli cells are present
1	Total absence of cells in tubular section

### Queen study

Unowned free-roaming queens were presented for ovariohysterectomy at a humane society in Oregon during August and September. Age was determined by dental eruption using Silver's chart.<sup>11</sup> The queen ages ranged from 2 - 6 months of age and were grouped for comparison: 2 - 4 months (n = 13) and 5 - 6 months (n = 4). After general anesthesia was induced, a routine ovariohysterectomy was performed. All queens recovered from surgery uneventfully. The total ovarian-uterine weights from queens were recorded. Both ovaries were hemi-sectioned, formalin-fixed, paraffin-embedded, cut into 6 µm sections, and 1 section from each ovary was stained with hematoxylin and eosin. Slides were analyzed using bright field microscopy at 200 x by a single observer (EB) blinded to the queen's age. Follicles were classified as primary, secondary, or tertiary<sup>13</sup> and the number of each follicle type was counted. The diameter of the largest tertiary (antral) follicle for each ovary was also recorded.

### Data analyses

Data were analyzed using GraphPad Prism, Version 8 (GraphPad Software Inc, La Jolla, CA). For all data, significance was defined as  $p < 0.05$ .

### Tom study 1

Diameters of 5 seminiferous tubules from each testis were averaged for each tom. Mean  $\pm$  SD seminiferous tubular diameter and spermatogenesis score for each age group (2 - 2.5, 3 - 4, 5 - 6, and > 6 months) were compared using one-way analysis of variance (ANOVA), followed by a Tukey's post hoc test. Presence of sperm in the seminiferous tubular lumen was compared among age groups (2 - 2.5, 3 - 4, 5 - 6, > 6 months) using a Chi-Square test.

## Tom Study 2

Presence of penile spines was compared between age groups (2 - 6 and > 6 months) using a Fisher's Exact test. Mean  $\pm$  SD percent normal sperm morphology for each age group (2 - 6 and > 6 months) was compared by an unpaired Student's t-test.

## Queen study

Linear regression was used to determine the effect of age on total ovarian-uterine weight. The percentage of each follicle classification (primary, secondary, tertiary) was compared by a Chi Square test. The largest tertiary follicle diameter for each age group (2 - 4 and 5 - 6 months) was compared by a Welch's t-test.

## Results

### Tom study 1

Seminiferous tubular diameters for 2 - 2.5, 3 - 4, 5 - 6, and > 6 months toms were  $88.1 \pm 10.9$ ,  $109.8 \pm 8.9$ ,  $142.2 \pm 16.9$ ,  $237.9 \pm 52.5 \mu\text{m}$ , respectively (Figure 1). Seminiferous tubular diameter was larger ( $p < 0.01$ ) in each successive age group. There was a 67% increase in seminiferous tubule diameter between toms 5 - 6 and > 6 months. Spermatogenesis and Leydig cell density scores were also different among age groups ( $p < 0.01$  and  $p = 0.01$ , respectively; Table 2).

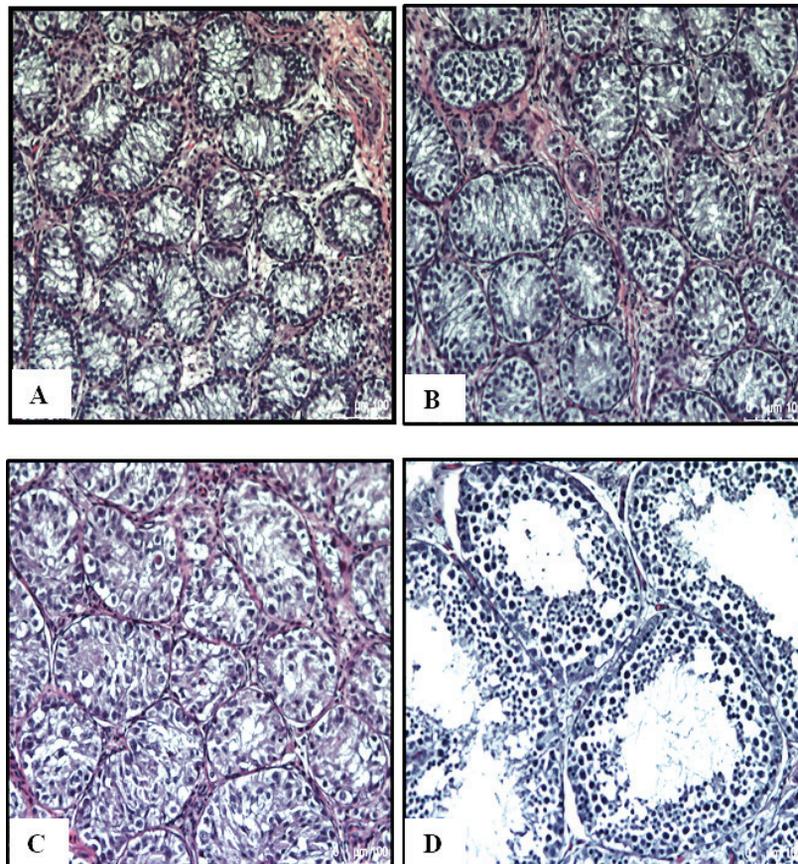
Spermatogenesis score in toms > 6 months was higher than in 2 - 2.5 or 3 - 4 months ( $p < 0.01$ ). In addition, Leydig cell density in was higher ( $p < 0.01$ ) in toms 2 - 2.5 months versus > 6 months.

### Tom study 2

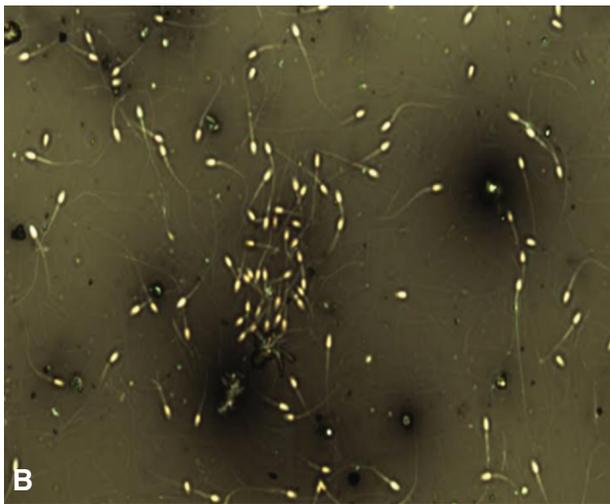
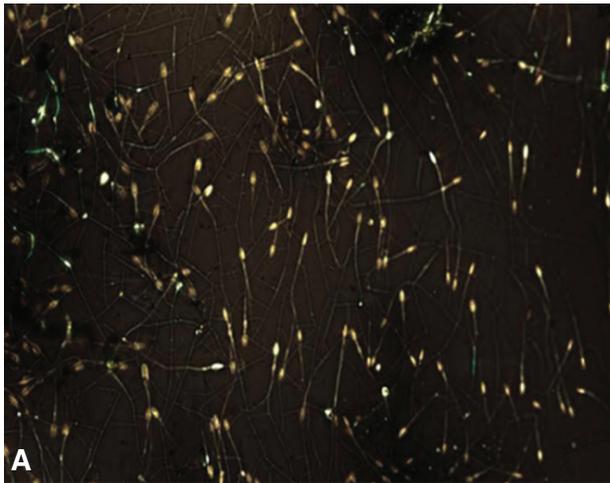
Eight of 13 (62%) toms aged 2 - 6 months and 12 of 16 (75%) toms aged > 6 months had penile spines ( $p = 0.07$ ). Sperm were present in vas deferens secretions from all toms examined, regardless of age. Percentage of normal sperm morphology (Figure 2) for samples with > 50 sperm per slide was not different ( $p = 0.39$ ) between toms aged 2 - 6 months ( $n = 11$ ,  $77 \pm 11\%$ ) and > 6 months ( $n = 9$ ,  $81 \pm 13\%$ ; Figure 2).

**Table 2.** Mean  $\pm$  SD spermatogenesis and Leydig cell density scores for unowned free-roaming toms. Within a column, means without a common superscript are different ( $p < 0.05$ ).

Age (months)	Spermatogenesis score	Leydig cell density score
2 - 2.5	$5.0 \pm 2.2^a$	$1.8 \pm 0.4^a$
3 - 4	$4.8 \pm 2.0^a$	$1.3 \pm 0.5^{a,b}$
5 - 6	$8.5 \pm 3.2^b$	$1.2 \pm 0.4^{a,b}$
> 6	$12.0 \pm 0.0^b$	$1.0 \pm 0.0^b$



**Figure 1.** Representative cross-sectional images of seminiferous tubules in toms of various ages. A: 2 - 2.5, B: 4 - 5, C: 5 - 6, and D: > 6 months. Magnification: 200 x. Hematoxylin and eosin stain. Bar = 100  $\mu\text{m}$ .



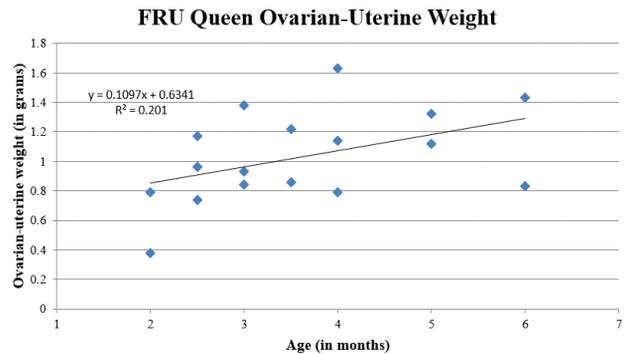
**Figure 2.** Representative sperm morphology photomicrographs prepared from vas deferens secretions from toms of 2 - 6 (A) and > 6 months of age (B). Magnification 1000 x. Eosin-nigrosin stain.

### Queen study

There was no association ( $R^2 = 0.20$ ,  $p = 0.07$ ) between total ovarian-uterine weight of queens and age (Figure 3). No differences ( $p = 0.08$ ) were observed between 2 - 4 and 5 - 6 months queens in the numbers of primary, secondary, or tertiary follicles (Table 3). Average ovarian-uterine weight observed in 4 - 6 months queens was  $1.18 \pm 0.31$  g. Diameter of largest tertiary follicle diameter did not differ ( $p = 0.14$ ) between queens 2 - 4 ( $581.6 \pm 53.7 \mu\text{m}$ ) and 5 - 6 months ( $469.4 \pm 113.9 \mu\text{m}$ ). A representative image of a tertiary follicle from a 2-month queen is illustrated (Figure 4).

### Discussion

In the current study, UFR toms aged 3 - 4 months had significantly wider seminiferous tubular diameter ( $109.8 \pm 8.89 \mu\text{m}$ ) compared to what has been reported ( $86 \mu\text{m}$ )<sup>14</sup> for domestic toms of similar ages. This finding was accompanied by most UFR toms 2 - 6 months and > 6 months having sperm in the vas deferens and a high percentage of these sperm with normal morphology ( $77 \pm 11\%$  and  $81 \pm 13\%$ , respectively).



**Figure 3.** Relationship of ovarian-uterine weight and age in queens (2 - 6 months old).

**Table 3.** Follicle classification (percent) in queens.

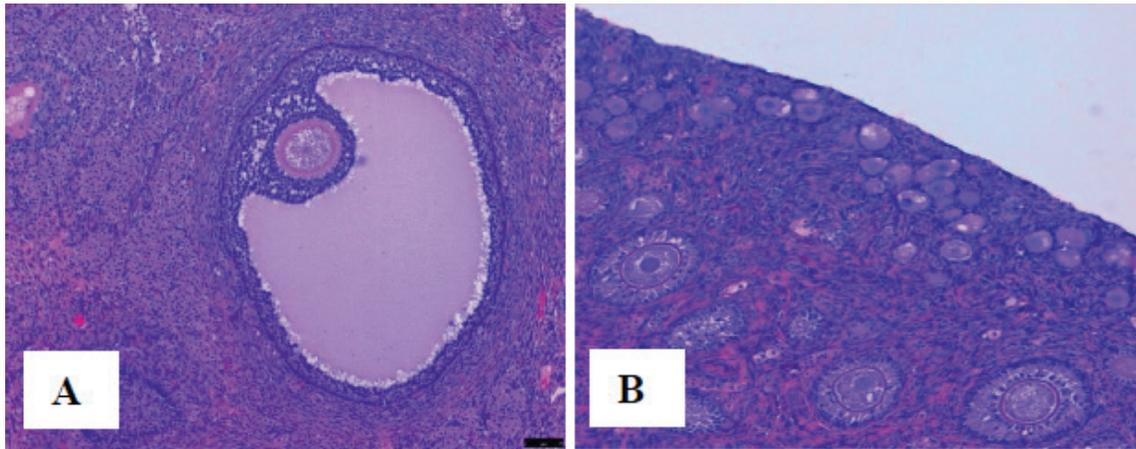
Age (months)	Primary	Secondary	Tertiary
2 - 4	47	23	30
5 - 6	35	20	45

Percentage of sperm with normal morphology from UFR cats in the current study was higher than that reported in fertile domestic cats (> 55 - 70%).<sup>15-17</sup> These data indicated that UFR toms as early as 2 months of age may be able to ejaculate sperm to achieve pregnancy. Together, these data supported the hypothesis that spermatogenesis is occurring at an earlier age in UFR toms (< 6 months) compared to what was reported in domestic toms (8 - 10 months).<sup>10</sup>

Although the 2 - 2.5 months UFR toms in the current study had the highest Leydig cell density score, the average score was 1.8 out of 3, demonstrating that these males only had scattered cells throughout the seminiferous tubules. It is unclear why the other age groups in the current study did not demonstrate higher densities, especially considering that previous studies have observed adult Leydig cells in domestic toms ~ 5 - 6 months of age.<sup>18</sup> However, growth of penile spines is positively correlated with androgen-secretion and androgen-dependent mating activity.<sup>19</sup> Therefore, the presence of penile spines is a good indicator that toms have the capacity to copulate and sire kittens. Fully matured penile spines are reported to not occur in domestic toms until ~ 8 months of age; however, they can first be detected ~ 6 - 7 months of age.<sup>20</sup> In the current study, 1 and 2 toms, 4 and 5 months old, respectively, had fully matured penile spines, suggesting that even at these young ages, toms may be mating with estrous queens.

In domestic nonpregnant queens, the average ovarian-uterine weight was reported to be 1.5 grams,<sup>21</sup> similar to weights observed in queens 4 - 6 months ( $1.18 \pm 0.31$  g) in our study. Young UFR queens had increased ovarian weights due to number and size of tertiary follicles as reported for prepubertal cats.<sup>22</sup>

Average largest tertiary follicle diameter in the current study was observed in queens 2 - 4 months. Although the tertiary follicles measured in the current study were smaller than reported for preovulatory follicles in queens (~ 3 mm), these findings suggest that UFR queens < 4 months age may have



**Figure 4.** Ovarian cross-section from a 2-month queen; note tertiary (antral) follicle. Magnification: 200 x. Hematoxylin and eosin stain.

the capacity to ovulate. This is supported by work in domestic queens that displayed pubertal estrus as early as 4.5 months.<sup>23</sup>

A limitation of the current study was that the exact age was not known due to the feral origins of the cats. For this reason, estimating age was based on dental eruption.<sup>11</sup> However, using dentition to determine the age of kittens is somewhat imprecise. For example, using Silver's chart, there is some overlap in ages, with eruption of permanent canine teeth occurring between months 5.5 - 6.5. For the purposes of the current study, cats with 4 fully erupted canine teeth were estimated to be > 6 months of age.

## Conclusion

Average lifespan of UFR cats is 2 years of age, with as many of 90% of kittens dying before 6 months of age.<sup>24</sup> These cats may have adapted to selective pressures of early-age mortality by collectively decreasing the age at which they can reproduce, ensuring the propagation of their genetic material before their death. Research presented in the current study supported a possible shift in puberty to a younger age in both male and female UFR cats. Toms < 6 months of age had penile spines with morphologically normal sperm in the vas deferens secretions. Queens as early as 2-4 months of age had small tertiary follicles. Together, this evidence explains why even with the amount of effort placed into TNR programs, humane societies in the United States still cannot surgically sterilize UFR cats fast enough to control their numbers.<sup>7</sup> To control UFR cat populations more effectively, sterilization efforts may need to include cats < 4 months of age.

## Care and use of animals

This research used tissues from animals that were not manipulated expressly for the purpose of obtaining those materials and therefore was exempt from IACUC review.

## Author contributions

Conceptualization, MK and EB; methodology, MK and EB; formal analysis, MK, EB, and KP; investigation, MK and EB; resources, MK; data curation, MK, EB, and KP; writing—original draft preparation, MK, EB, KP, and DB; writing—review and editing, MK, EB, KP, and DB; supervision, MK; project

administration, MK. All authors have read and agreed to the published version of the manuscript.

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## Conflict of Interest

Authors declare no conflict of interest.

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