AN ABSTRACT OF THE THESIS OF

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The purpose of this thesis was to determine if an underlying biological cause exists for the exuberant reproductive success in free-roaming unowned (FRU) cats. The hypothesis for this thesis was that FRU tom and queen cats have reproductively adapted to man-made sterilization efforts by lowering the age at which they enter puberty. For domestic cats, puberty is reported to occur around 8 months of age. Cats were presented for surgical sterilization at either a feral cat clinic or at a local Humane Society during August-October 2014 and 2015. Age was determined by records provided from feral cat colony managers and confirmed with dental eruption patterns. The age groups for tom cats were: 2-2.5 months (weanling; n=6), 3-4 months (juvenile; n=6), 5-6 months (pubertal; n=6), and 12-24 months (adult; n=6). Queens were grouped by age (<4 months (pet n=5, FRU n=10) and 4-6 months (pet n=2, FRU n=7)). For tom cats, the penis was evaluated to determine if spines were present and the contents from both vasa deferentes were milked onto a microscope slide, mixed with eosin-nigrosin stain, spread with a spreader slide, allowed to air dry and evaluated at 1000X. The percentage of sperm with normal morphology was determined after evaluating 100 sperm/slide. Testicles were hemi-sectioned, formalin-fixed, paraffin-embedded, cut into sections (6 µm), stained with hematoxylin and eosin (H&E), and evaluated at 200X for evidence of spermatogenesis and measurement of seminiferous tubule diameter. For queens, the total ovarian uterine weights from FRU queens were also recorded. Ovaries were hemi-sectioned, formalin-fixed, paraffin-embedded, cut into sections (6 µm), stained with H&E, and evaluated at 200X. Follicles were counted and classified as primary, secondary, or tertiary. More adult toms (16/16) than juvenile toms (4/13) had penile spines ($p<0.05$). Mean±SD morphologically normal sperm for the juvenile and adult tom cats was not significantly different (77±11% and 81±13%, respectively). Evidence of spermatogenesis in weanling, juvenile, pubertal, and adult toms was 0%, 17%, 67%, and 100%, respectively ($p<0.05$ between successive age groups). The seminiferous tubular diameter was significantly larger in each successive age group (weanlings 88.10±10.88 µm; juveniles 109.8±8.89 µm; pubertal 142.2±16.89 µm; adult 237.90±52.45 µm). FRU queen cats under 4 months old had more tertiary follicles compared to owned cats under 4 months of age (33% and 17%, respectively; $p<0.05$). Total ovarian uterine weights were significantly higher in 4-6 month old FRU queens compared to under 4 months (1.18±0.31 g vs 0.93±0.28 g, respectively). These observations provide an explanation for why TNR efforts to reduce FRU cat populations have not successful. Selective pressures and a significantly shortened life span may be factors contributing to this finding.

Key Words: Feral, Overpopulation, Puberty, Queen, Tom

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I understand that my project will become part of the permanent collection of Oregon State University, University Honors College. My signature below authorizes release of my project to any reader upon request.

Ellie Bohrer, Author

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CHAPTER I. INTRODUCTION

I.A. FRU Cat Definition

A feral animal is any animal living in the wild that descended from domesticated individuals. Feral animals can be found across the globe, with many different kinds of previously domesticated animals roaming and living as part of the ecology. Some examples of feral animals include feral horses, donkeys, pigs, goats, cows, mongeese, dogs, and cats. Although these animals are considered a part of the community, they are non-native species that can cause damage to ecological structures, and many are accordingly classified as invasive species. There are many different definitions of what a feral cat is. In fact, it is a highly controversial topic among scientists, veterinarians, and cat colony managers. A study by Gosling et al (2013) demonstrated that there is a huge variation in what people believe a truly feral cat to be. In this study, a mixed methods questionnaire was distributed amongst rescue workers and veterinary surgeons in the United Kingdom. Although the definitions varied from person to person, a practical definition of a feral cat was formed (Gosling et al, 2013):

1. A feral cat is unapproachable in its free-roaming environment and is capable of surviving with or without direct human intervention.
2. When trapped, a feral cat will either display defensive tactics, or cower and try to hide.
3. When released into a confined space, it will not be possible to handle the cat.

Another major argument among the scientific and animal welfare community is the distinction between a community cat and a feral cat. According to the Humane Society of America, community cats are individuals that live their lives outdoors, which encompass friendly stray and abandoned cats (AVMA, 2012). According to this definition, the only difference between community cats and feral cats is that feral cats are not socialized. Feral and community-owned cats are often referred to as living in a colony. A colony is defined as a group of three or more sexually mature (aged five months or more) cats living and feeding in close proximity (Slater et al, 2005). It is important to note that some studies refer to colonies outside of these defined parameters (i.e. include kittens and cats under five months of age who are living with the other colony members).

For the purpose of this thesis research, feral and community-owned cats were classified under the umbrella term of free-roaming unowned cats (FRU). However, comparisons may be drawn between feral and community-owned cats. Also for the purpose of this thesis research, feral cats were defined as having the major traits described by Gosling et al (2013), which include: being unapproachable, unmanageable, and capable of surviving without direct human intervention. Community-owned cat were cats that have traits similar to feral cats and they are also free-roaming, but the main distinction between these two groups is that community-owned cats cannot survive without direct human intervention. This includes free-roaming cats that are actively fed and provided water by people in the community. In this thesis research, comparisons were made between pet cats and FRU cats. A pet cat was defined as an animal that is completely cared for by human intervention. This includes providing the cat with food,

water, and shelter. It is important to note, however, that pet cats may also spend a large amount of time outdoors or potentially live outdoors. These cats may even obtain external food sources such as catching prey, however, their main food source is provided by humans. It is important to keep in mind that many individuals have differing opinions on what these classifications are, but for the purposes of this thesis research, these were the definitions used.

I.B. Problems Associated with FRU Cats

I.B.1. Epidemiological Problems

One deleterious capability of FRU cats is their ability to serve as vectors for numerous diseases. As FRU cats are typically not vaccinated (unless done so by a Trap-Neuter-Release program or otherwise), they can serve as reservoirs for many diseases. These diseases can affect many other species, including pets and even humans. Feline panleukopenia virus (FPV) is a highly contagious and often fatal viral disease of cats. Cats can contract the virus oronasally by exposure to infected animals, their feces, secretions, or contaminated fomites (such as food bowls, litter pans, bedding, cages, etc). Most FRU cats are thought to be exposed to the virus during their first year of life, with the prevalence of infection at 79% (Coman et al, 1981). FRU cats can serve as reservoirs for both feline leukemia virus (FeLV) and feline immunodeficiency virus (FIV), with infectious individuals shedding high amounts of the virus in their body fluids. Feline Leukemia Virus (FeLV) and Feline Immunodeficiency Virus (FIV) are common infectious diseases of FRU cats. FeLV is typically transmitted via contact with body fluids including saliva, nasal secretions, urine, feces, and mother's milk. FIV is typically

transmitted via bite wounds, and in rare cases can be transmitted from mother to kitten during passage through the birth canal, or by the kitten ingesting infected milk. Lee et al (2002) reported the overall prevalence of FeLV and FIV within FRU cats to be 4.3% and 3.5%, respectively.

FRU cats also have the ability to transmit zoonotic diseases. This is a concern for many communities as human health can be jeopardized by contact with FRU cats that carry these diseases. FRU cats can serve as a rabies vector via their contact with other unvaccinated animals, as well as contact with other wildlife vectors. Unvaccinated cats account for a substantial proportion (~16%) of postexposure prophylaxis administered in the United States (PEP) (Hensley, 1998; Moore et al, 2000; Christian et al, 2009).
Because rabies vaccination must be repeated every 1-3 years, FRU cats vaccinated in conjunction with TNR programs still serve as vectors after their immunity wanes (Richards et al, 2006; Foley et al, 2005).

One of the most familiar feline to human zoonotic diseases is *Toxoplasma gondii*, a tissue protozoan. At least 30% of cats (including FRU cats) and humans have previously been exposed to *T. gondii* (Reif, 1980; Danner et al, 2007). Cats are the definitive host of *T. gondii*, as they are the only species known to complete the sexual phase (enteroepithelial cycle) (Lappin, 1993). The sexual phase results in the passage of unsporulated oocysts in the feces, which can sporulate in the environment when in the presence of oxygen. Oocysts can live in the environment for months to years. Humans can be infected with *T. gondii* by ingesting sporulated oocysts or in tissue cysts (Lappin, 1993). In general, most immunocompetent individuals do not develop clinical signs after primary exposure. However, in some cases, individuals may present with signs similar to

infectious mononucleosis including fever, lymphadenopathy, and malaise (Lappin, 1993). Primary exposure of the mother during pregnancy during the first two trimesters commonly leads to infection of at least 50% of fetuses, with severe clinical diseases in at least 10% of fetuses (Elliot et al, 1986).

I.B.2. Ecological Problems

Another risk associated with FRU cats is their ability to cause destruction to native ecology. The magnitude of destruction by these animals causes many governments and natural organizations to classify FRU cats as an invasive species. These animals are considered so invasive in nature that FRU cats have been listed among the 100 worst non-native invasive species in the world (Lowe et al, 2000). It has been estimated that FRU cats on islands have caused or contributed to 33 (14%) of the modern bird, mammal, and reptile extinctions recorded by the International Union for Conservation of Nature (IUCN) Red List (Medina et al, 2011). Cats in the contiguous United States kill approximately 2.4 billion birds annually. Of these 2.4 billion birds killed, approximately 69% of this mortality is caused by FRU cats. The reason for the increased predation estimate amongst FRU cats is primarily due to the notion FRU cats have a predation rate that is three times greater than pet cats (Loss et al, 2013). It is also worth noting that an estimated 478 million reptiles and 173 million amphibians are killed by cats in the contiguous United States each year (Loss et al, 2013). The estimated annual mammal mortality rate caused by cats in the contiguous United States is 12.3 billion. Of this mortality, 89% is caused by FRU cats (Loss et al, 2013). These findings show that individual FRU cats kill on average 200 mammals per year (Hawkins et al, 2004). FRU

cat predation can cause destruction of native populations, resulting in small mammal extinction. A particularly well-documented case is that of the pallid beach mouse (*P. p. decoloratus*). *P. p. decoloratus* went extinct around 1959. Extensive survey sampling at the type locality for the pallid beach mouse found a high density of FRU cats (Humphrey and Barbour, 1981). Extinction was probably a result of FRU cat predation. It is important to note that FRU and outdoor pet cats can all contribute to the predation of a particular species.

Not only do FRU cats affect terrestrial organisms, their damaging nature even leeches into marine systems. Recent studies have found high levels of *T. gondii* in marine ecosystems, in particular, southern sea otters (*Enhydra lutris nereis*) and Hawaiian monk seals (*Monachus schauinslandi*) (Gibson et al, 2011). As previously mentioned, felines are the only recognized definitive hosts of *T. gondii*. This parasite spreads in marine ecosystems when cats shed *T. gondii* oocysts in their feces, and the feces are transported into marine systems via freshwater runoff. Toxoplasmosis is a major cause of mortality and contributor to the slow rate of population recovery for southern sea otters in California (Conrad et al, 2005). Toxoplasmosis infection in monk seals can be deadly, with NOAA reporting several individuals of this endangered species succumbing to *T. gondii* infection (NOAA Fisheries, 2013). In fish and other marine mammals, *T. gondii* can create parasitic cysts in muscle tissue and organs.

I.C. Methods for Controlling FRU Cat Population

I.C.1 FRU Cat Population Numbers

It has been hypothesized that the number of FRU cats likely rivals or exceeds the number of pet cats in the world. Current estimates place the number of FRU cats in the United States alone at 60 million individuals. The projected global population of FRU cats is estimated to be about 158 million individuals (Batson, 2008). According to the Humane Society of America, only about 2% of FRU cats are spayed or neutered. Taking this statistic into account, the ability of these animals to breed unhindered by sterilization measures is virtually unlimited. Although the number of FRU cats rivals that of pet cats, FRU cats produce 80% of the kittens born in the United States each year (Humane Society, N.d.). These statistics may help to explain why extreme overpopulation is observed in many FRU cat colonies. Interestingly, only 10-12% of people in the United States provide food for FRU cats (Humane Society, N.d.). This indicates that one individual food resource provided by a human may be enough to sustain multiple individuals.

I.C.2. Current Population Control Strategies

There are several FRU cat population control strategies in use today. These include euthanasia, destroy on site, trap, remove and euthanize, trap, remove/relocate, trap, neuter, release (TNR), and non-surgical contraception. The most publicly supported control program is TNR, which is widely stated throughout the United States, as well as other countries such as Great Britain, Canada, the Netherlands, and Denmark (Neville and Remfry, 1984). The goal of TNR programs is to stabilize or reduce a local population by sterilization. It is assumed that because FRU cats are less likely to move in to populate a vacated space, there will be natural attrition of the returned sterilized cats. However, it

has been documented that FRU cats, especially males, move between colonies (Levy et al, 2003). In general, TNR involves the humane trapping of FRU cats, sterilization (by a veterinarian), permanent identification of sterilization status (ear tip), vaccination for rabies (other vaccines such as FPV and FeLV may be administered depending on the program and municipality), and release back to the original trapping location. Very sick and injured cats are humanely euthanized on site (Robertson, 2007).

There is some debate as to the efficacy of TNR programs in controlling FRU cat populations. Successful TNR campaigns in Florida, and Rome, Italy, have been reported (Hughes et al, 2002; Natoli et al, 2006). However, for every positive TNR campaign, multiple failures are reported. Even with the advent of TNR programs, humane organizations throughout the United States still can't surgically sterilize FRU cats fast enough to control their numbers (Grimm, 2009). Many argue that TNR programs do not control for the effects of important social behaviors in FRU cats, including dominance (Mirmovitch, 1995). Dominance status is complex and may differ in different populations. Dominant males that are castrated in a TNR program become sexually inactive and are subsequently replaced in the breeding hierarchy by the next most dominant male, so unless every male in a colony can be captured and castrated, continued reproduction with maintenance of population size is inevitable (McCarthy, 2013). Vasectomy has been proposed as a solution, as vasectomy does not alter a male cat's sexual drive or social status. Vasectomized cats maintain their position in the breeding hierarchy, compete with other males for females as before surgery, prevent less dominant males from breeding, and continue to copulate (however unsuccessfully) (Pineda and Dooley, 1984).

Various estimates place castration costs of FRU cats in TNR programs around $70.77 ± $11.75 per cat nationally (Nutter and Stoskopf, 2004). With 60 million FRU cats in the United States alone, in order to effectively sterilize all individuals, it would cost approximately 4.2 billion dollars. TNR programs not only cost communities in the United States hundreds of thousands of dollars, but also require a very large volunteer effort on the part of local advocates and veterinarians. These expenses are one major reason why it is important to understand why FRU cats are so reproductively successful. Once their specific biology is better understood, sterilization efforts can incorporate these principles to target populations more appropriately and save time and money in doing so.

I.D. Feline Reproductive Anatomy and Physiology

Cats are polyestrous reflex-induced ovulators, which signifies that the act of mating induces ovulation in the female. Compared to other mammals, domestic cats have a relatively short gestation period of about 66 days (Tsutsui and Stabenfeldt, 1993). This short gestation period coupled with an average litter size of three kittens can lead to exponential growth in a population not subjected to control measures (Nutter et al, 2004). Breeding seasons are photoperiod-controlled, with a normal breeding season usually commencing in January and having the capacity to last until October in the Northern hemisphere (Tsutsui et al, 2004). This period can vary based on local climate and light exposure (Levya et al, 1989). In order to determine whether the FRU cat population is collectively reaching puberty sooner than what has been reported for domestic cats, we must identify the age at which FRU cats are considered to be reproductively viable. There

are a number of anatomical and physiological indicators that can be used to determine sexual maturity in male (tom) and female (queen) cats.

I.D.1. Tom Cat Anatomy and Physiology

Sperm are produced, stored, and delivered by the male reproductive system. The main structures of the male reproductive system include the testes, epididymis, vas deferens, urethra, os penis, and penis. The primary axis of sexual maturity in toms revolves around the production of the steroid hormone testosterone. Testosterone is produced in the testes, which causes the secondary sex characteristics associated with puberty. Puberty in domestic toms generally occurs around 8 months of age and coincides with the appearance of several physiological and anatomical indicators that can be examined (Tiptanavattana et al, 2014).

An external morphological indicator of puberty in toms is the presence of spines on the penis. Penile spines are relatively large, horny spines of papillae located on the penis in male felines. The corpus cavernosum glandis of the feline penis contains 100-200 cornified spines, which can vary in overall size and shape. Penile spines are sensitive to androgens (i.e. testosterone), and they are the only known external indicators of the level of male hormones in cats. Studies have shown that penile spines increase in size as androgen levels rise, and concurrently decrease in size as androgen levels fall (Aronson and Cooper, 1967). Thus, the size and appearance of the cornified spines can be used as an indirect measurement of testosterone, and therefore sexual maturity in the cat. Fully matured penile spines are reported to occur around 8 months of age in the domestic tom, but they can first be detected around 6-7 months of age (Stabenfeldt and Shille, 1980). In

the sexually mature adult, the prepuce of the penis is attached to the proximal end of the glans at its junction with the shaft of the penis. There are approximately 120 to 150 backward pointing spines that encircle the glans. The diameters at the base of the spines measure similarly from 0.7 mm proximally to 0.1 mm distally (Aronson and Cooper, 1967). Although the exact function of penile spines is not clearly understood, it has been hypothesized that the spines are used to induce ovulation in the female (Goodrowe et al, 1989).

The most important activity of the testes is the production of male gametes (Siemieniuch et al, 2007). The second activity of the testes is the production and release of hormones (Leme et al, 2002). The testes are composed of two morphologically and functionally different compartments, the seminiferous tubules and the interstitial tissue. The production of gametes can be observed in the testicle in the seminiferous tubules. The seminiferous tubules contain two distinct cell populations: the Sertoli cells and the germ cells. The Sertoli cells are somatic cells that play an essential role in the development of the other population, the germ cells, from which spermatozoa arise.

The interstitial tissue of the testes contains the Leydig cells, which produce androgens. The two testicular compartments are functionally interdependent; androgens are essential for Sertoli cell maturation and spermatogenesis, and Leydig cell development and function are modulated by various factors secreted by the seminiferous tubules (Rey, 1999). Spermatogenesis is the process by which spermatozoa are produced from primordial germ cells via mitosis and meiosis. During the process, primordial germ cells are transformed into four genetically different mature sperm cells in the testes. Spermatogenesis is a complex yet highly organized process that encompasses several

observable sperm cell stages, which can be classified by both the morphological changes observed in the spermatozoa and the location of the spermatozoa in the seminiferous tubule (Franca et al, 2003). As the sperm mature, they can be observed in different parts of the seminiferous tubule. Therefore, the arrangement and location of sperm in the seminiferous tubule can be used to classify the maturity level of the sperm (Courot et al, 1970; Berndtson, 1977). The bulk of the initial spermatozoa maturation in mammals can be observed in the seminiferous tubule epithelium. Sperm mature from spermatogonia in the outer epithelium of the seminiferous tubule and progress in maturation stage through the epithelium until they reach the lumen, where mature sperm may be observed. Therefore, the presence of spermatozoa in the lumen of seminiferous tubules is a major indicator of sexual maturity. This is reported to occur around 8 months of age in the domestic tom (Tiptanavanna et al, 2014). After the sperm are ejected into the seminiferous tubule lumen, they are further modified and matured via many chemical processes in the epididymis. Seminiferous tubule diameter size has also been correlated with sexual maturity in rats, humans, and other mammals. In bulls, the considerable increase in tubular diameter size is due to a massive increase in the proliferation of germ cells. A similar trend has also been observed in domestic toms. In a study performed by Sanchez et al (1993), seminiferous tubule diameters were examined via histomorphometry and a mean diameter value produced for different ages of cats, ranging from 1 month to 1 year (infant through adult period). In infant cats (1 month to 5 months of age), the average tubule diameter was 86 µm. The peak seminiferous tubule diameter size was observed in adult cats (8 months or more of age), with an average diameter of 220 µm. Overall, as the age of the animal increased, the average tubule diameter also

increased, with the largest tubule diameters being observed around 8 months of age (Sanchez et al, 1993b). This age coincides with the age associated with the presence of spermatozoa in the seminiferous tubule lumen, another indicator of sexual maturity. Thus, seminiferous tubule diameter can be used as an indicator of sexual maturity in felines.

Sperm morphology is important to analyze as a factor of fertility in males as specific morphological characteristics of the sperm can dictate whether or not spermatozoa in the ejaculate can properly fertilize an egg. Various parts of the sperm are integral for the transportation of the spermatozoa to the egg (i.e. tail) and the fusion with the zona pellucida (i.e. acrosome), the first step in the process of fertilization. In a small group of research colony cats, it was found that males with less than 40% morphologically normal spermatozoa obtained lower zona pellucida penetration rates than did cats with greater than 60% normal spermatozoa, one such example of the importance of correct sperm morphology (Howard et al, 1993). Percentage of morphologically normal and abnormal sperm in the ejaculate is determined by examining 200 sperm using phase-contrast microscopy or by routine light microscopy following staining with Diff-Quik or eosin-nigrosin stain (Johnston et al, 2001). There are many different sperm morphological abnormalities to consider in semen analysis. Some of the most common morphologic abnormalities of feline sperm include macroencephaly, microencephaly, double head, double tail, proximally coiled tail, bent mid-piece, proximal and distal retained cytoplasmic droplet, detached head, and bent tail (Howard et al, 1990). An acceptable overall normal percentage of spermatozoa morphology has been debated. It has been claimed that domestic cats produce ejaculates with greater than 60%

morphologically normal spermatozoa (Wildt et al, 1983). However, some studies show that the average domestic cat may express a considerably lower percentage of normal spermatozoa. In a retrospective study performed by Axner and Linde Forsberg (2007), the median percentage of normal spermatozoa in a population of random domestic cats was 56.1% during the reproductive season. Thus, there is some discrepancy between a cut-off for normospermic cats and teratospermic cats.

Semen collection for sperm analysis can be performed via several methods. The most common methods to collect sperm in the cat are by electroejaculation or via artificial vagina. Both techniques enable collection of whole semen with good quality. Spermatozoa can also be collected by squeezing or slicing of the epididymis, which is frequently used for research with the advantage to obtain tissues after routine orchiectomy. However, epididymal samples generally demonstrate greater proportions of abnormal spermatozoa (36%) than do ejaculated cells (26%).

The production of testosterone and subsequent sexual characteristics are hypothesized to vary with the seasons in domestic toms. Testicular histology studies reveal the presence of sperm in the seminiferous tubules during all four seasons of the year. However, testicular weights seem to be positively correlated with increasing length of photoperiod. A link also appears to exist between plasma testosterone concentrations and photoperiod (Kirkpatrick, 1985). The assumption of a physiological relationship between testosterone concentrations and testicular function suggest some degree of photoperiodic control of the testes in tom cats (Joffre, 1977). The percent normal sperm morphology that a particular tom expresses seems to also vary with photoperiod. Several studies have found that the mean percentage of morphologically normal spermatozoa is

significantly higher during the reproductive season than during the non-reproductive season. The mean percentages of proximal droplets, distal droplets, detached heads, acrosomal abnormalities, and sperm tail abnormalities have found to be higher during the non-reproductive season. Acrosomal abnormalities seem to be particularly affected by seasonality (Axner and Linde Forsberg, 2007). In a study performed by Blottner and Jewgenow (2007), FRU cats in Berlin, Germany were captured and their testes analyzed to investigate the potential effect of seasonality on sperm and testosterone production. The study suggests that there may be moderate seasonal changes in the quantity of sperm and testosterone production, and a distinct seasonal influence on sperm function (i.e. motility) in FRU cats (Blottner and Jewgenow, 2007).

I.D.2. Queen Anatomy and Physiology

Female gametes (oocytes) are produced by the female reproductive system. The main anatomical structures in the female feline include the ovaries, uterine tube and uterus, vagina, vestibule, and vulva. Estrogen is the primary female sex hormone and is responsible for the development and regulation of the female reproductive system and associated secondary sex characteristics. Other hormones associated with the feline reproductive cycle include follicle stimulating hormone (FSH) which initiates follicular growth (specifically granulosa cells), luteinizing hormone (LH) which triggers the release oocytes, and progesterone, which is involved in pregnancy and embryogenesis. Puberty in domestic queens has been reported to occur between the ages of 8 and 10 months of age. However, the onset of puberty may be affected by the breed of the individual, the

breeding environment, as well as the timing of birth relative to the photoperiod-controlled annual breeding season (Jemmett and Evans, 1977).

Unlike canines, female cats do not demonstrate an estrous cycle. Queens typically show continuous or near continuous estrus throughout a breeding season unless the cycle is interrupted by pregnancy, pseudopregnancy, or illness (Colby, 1980). The queen can be bred at any time during estrus (Tsutstui and Stabenfeldt, 1993). Anestrus typically begins in late October and ends in late December. Individual variability is common, although shortening day lengths and excessive heat are factors that may induce the onset of anestrus (Leyva et al, 1989).

It has long been known that estrogen has a large effect on the growth and development of the female reproductive organs. Studies have shown that estrogen production has a positive correlation with increasing uterine weight in rats and other mammals (Medlock, 1994). Therefore, uterine weight can be used as an indirect measure of estrogen and therefore sexual maturity in the cat. Size of the uterus depends on the size, age, and parity of the cat and the phase of the estrous cycle or stage of pregnancy. In non-pregnant adult domestic cats, the uterus weighs about 1.5 grams (Latimer, 1939).

The gross and histologic appearance of the ovary varies with the stage of the estrous cycle (Wildt, 1980). During anestrus the surface of the ovary is smooth, and small follicles are visible histologically. As the follicular phase (estrus) approaches, three to seven follicles enlarge, and the rest undergo atresia. Most follicular development occurs in the two-day interval just prior to the onset of estrus behavior (Wildt and Seager, 1978). Within the ovary, isolated follicular units composed of specialized cells surround each oocyte. The follicle aids in the development of a healthy oocyte, which upon appropriate

endocrine signaling, will be ovulated, and perhaps fertilized. Follicles mature through the complicated process known as folliculogenesis, which is dependent on classic endocrine signaling within the hypothalamic pituitary-gonadal axis (Bristol-Gould and Woodruff, 2006). Cat ovarian follicles resemble follicles found in other mammalian species, although the major morphological distinction between cats and rodents is the clear abundance of primordial follicle observed throughout the ovarian cortex (Bristol and Woodruff, 2004). The ovarian follicle consists of multiple differentiating cell types, while the fundamental core is composed of an oocyte enveloped by granulosa cells (Figure I.1).

Primordial follicles are the smallest follicles and are distinctly observable beneath the tunica albuginea. The oocyte ranges from 20-30 μm in diameter. One to eight flattened or squamous pre-granulosa cells directly surround the oocyte (Bristol-Gould and Woodruff, 2006). Primary follicles are larger than primordial follicles, at about 100 μm in diameter. Multiple layers of granulosa cells are present, and a fully grown oocyte is clearly observable in the center of the follicle. At this stage, the zona pellucida is easily detected, in addition to a basement membrane separating the surrounding layer of granulosa cells from the ovarian stroma. At least two layers of granulosa cells are apparent (Bristol-Gould and Woodruff, 2006).

Secondary follicles range from 200-300 μm in diameter. Typically, many layers of granulosa cells are apparent and a theca cell layer is now deposited opposite from the basement membrane. Tertiary (or antral) follicles are generally 400-1000 μm in size. The main feature is the appearance of a fluid-filled antrum. The follicles are surrounded by two to three layers of thecal cells. More mature tertiary follicles contain a larger antral

space. The oocytes are usually positioned in the cortex near the periphery of the ovary awaiting an adequate LH stimulus.

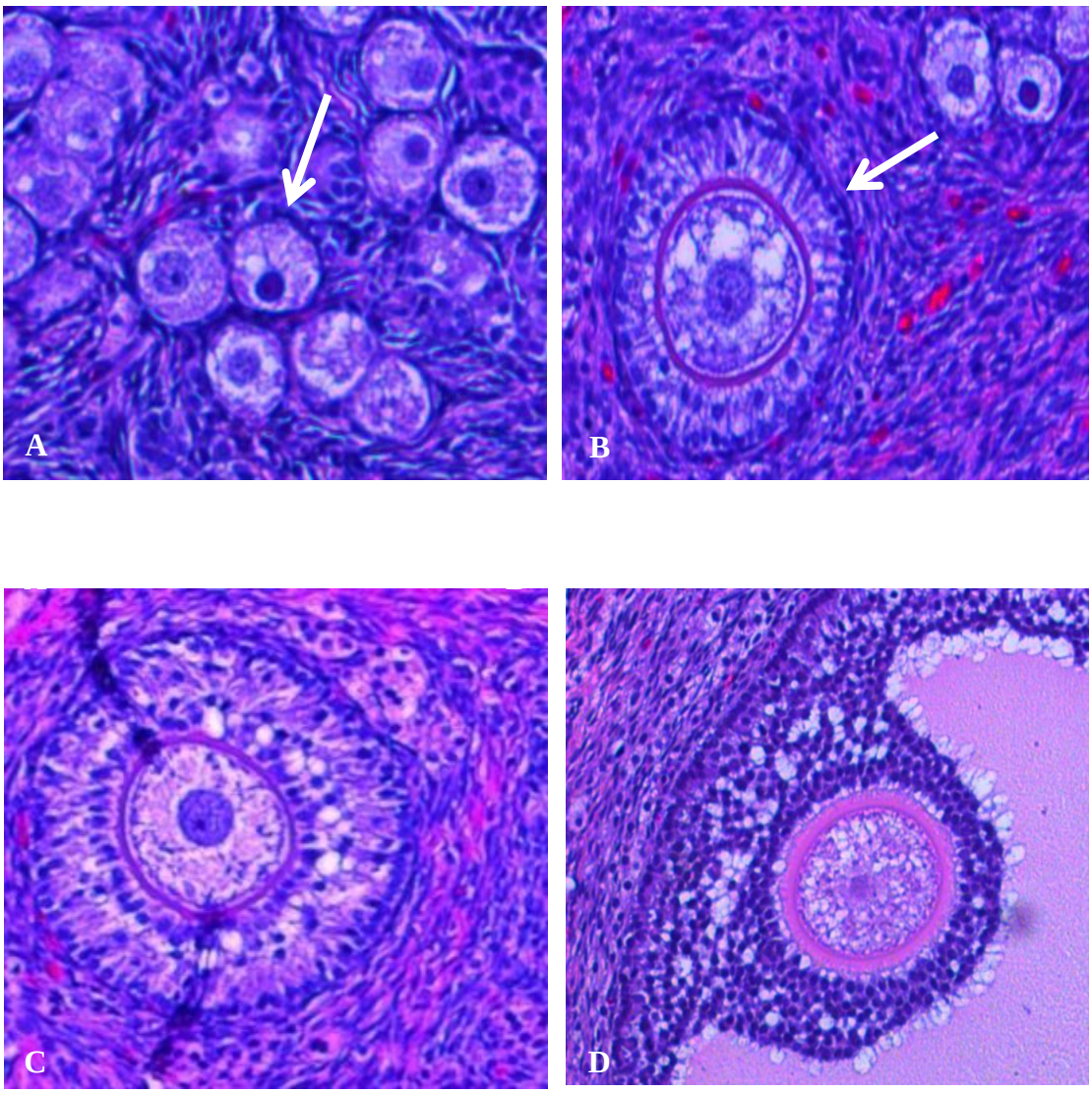

Figure I.1. During folliculogenesis, the ovarian follicle consists of multiple differentiating cell types, while the fundamental core is composed of an oocyte enveloped by granulosa cells. Representative follicle types include primordial (A), primary (B), secondary (C), and tertiary (D).

In a study performed by Uchikura et al (2010), the ovaries from 93 pre-pubertal FRU cats were examined to evaluate follicular development and oocyte quality in pre-pubertal cats. The ovarian weight of cats in the study increased rapidly until their body weight reached approximately 1000g (~2.2 lbs). This corresponded to an estimated age of 100 days, and afterward increased slowly. The increase in ovarian weight was accompanied by the increase in the number of antral follicles and in the size of the secondary and tertiary follicles. Thus, it appears that the increase in ovarian weight resulted, at least in part, was due to the increase in the number and size of tertiary follicles (Uchikura et al, 2010). This finding is similar to what has been reported in other species (Mahdi and Kahlilli, 2008; Desjardins and Hafs, 1969).

There is evidence in a variety of mammals that photoperiod influences reproductive processes through the hypothalamo-hypophysial-gonadal axis via the pineal gland and its main hormone, melatonin (Reiter, 1980). A period of extended daylight (more than 12 hours per day) induces estrus in the cat (Hurni, 1981). It is recommended that the lighting cycle should be 14 hours or more for cat breeding (Root et al, 1995). In cats, when the photoperiod is shortened, melatonin and PRL secretion are enhanced, reducing ovarian function (Leyva et al, 1989). It has been suggested that there is no relationship between the number of estrous cycles and the duration of the breeding season, suggesting that the interestrus interval differs noticeably among individual animals (Tsutsui et al, 2004). Individual acclimation to photoperiod has led to spontaneous ovulation in a high proportion of cats. Gudermuth et al (1997) reported that a high proportion of cats showed spontaneous ovulation under group acclimation (Gudermuth et al, 1997).

CHAPTER II. MATERIALS AND METHODS

Two observational groups were assembled for this study on the reproductive capacity of FRU cats. The first study focused on tom cats and kittens, whereas second study focused on queens and kittens. In order to control for testosterone and estrogen changes due to seasonality, all tissues were collected during the months of August-October.

II.A. FRU Tom Cats

FRU tom cats were presented for castration at a feral cat neutering clinic in Corvallis, Oregon, during the month of September 2013. The individuals in this study were categorized into two age groups. Toms 2-6 months of age were defined as a juvenile (n=13). Individuals over 6 months of age were classified as adults (n=16).

After general anesthesia was induced in the individual, a physical examination of the penis was performed to determine whether penile spines were present. The presence of penile spines was scored on a numerical scale, with a score of zero being defined as not present, and a score of one representing fully developed penile spines.

Next, the hair on the scrotum was clipped, and the skin was aseptically prepared for surgery. A routine open castration was performed, and both testicles were removed for analysis. All toms recovered from surgery uneventfully. Fluid contents from both vas deferens were milked by hand onto a microscope slide. These secretions were mixed with one drop of eosin-nigrosin morphology stain, spread with a spreader slide, and allowed to air dry.

The sperm morphology slides were evaluated by the same observer (Bohrer, 2015), blinded to the age group of the individual. Microscopic analysis was performed using bright field microscopy, under oil immersion (1000X), using a Leica inverted stage fluorescence microscope with imaging. The percentage of spermatozoa with normal morphology was determined for each individual.

The presence of penile spines was compared using a Chi-Square test. The percent normal sperm morphology was compared using a Student's t test. Significance was defined as $p<0.05$.

An additional group was assembled for this study to examine spermatogenesis in FRU toms. FRU toms were presented for castration at a Humane Society in Corvallis, Oregon, during the months of August through October 2014. Testicles from 24 individuals were collected to be analyzed via histology for the presence of spermatogenesis. The age groups in the testicular histology study were as follows: 2-2.5 months (weanling; n=6), 3-4 months (juvenile; n=6), 5-6 months (pubertal; n=6), and 12-24 months (adult; n=6).

General anesthesia was induced, the hair on the scrotum was clipped, and the skin was aseptically prepared for surgery. A routine open castration was performed, and both testicles from each cat were obtained. All toms recovered from surgery uneventfully. Tissues were formalin fixed, paraffin-embedded, cut into 6 μm sections, and stained with hematoxylin and eosin.

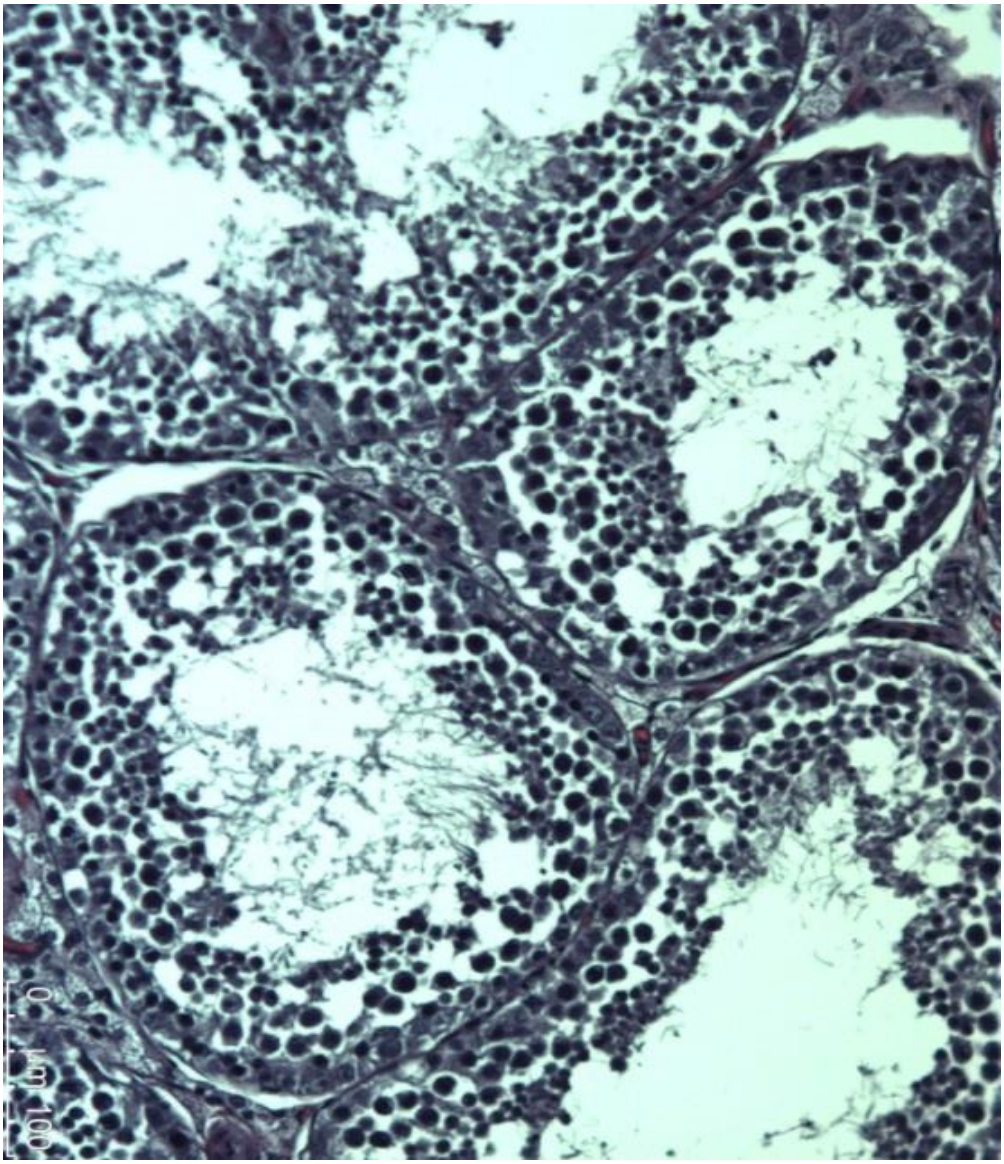

Figure II.1. Presence of spermatozoa in the seminiferous tubule lumen of a FRU tom cat (Bohrer et al. Histologic and morphometric evaluation of testes of FRU tom kittens and cats. Society for Theriogenology Annual Conference Proceedings 2015 (abstract)).

Slides were evaluated via histology by a single observer (Bohrer, 2015), blinded to the age group of the individual. This analysis was performed using bright field microscopy at 200X, using a Leica inverted stage fluorescence microscope with imaging. Evidence of spermatogenesis was determined on the basis of presence of spermatozoa in the seminiferous tubule lumen (see Figure II.1). Perpendicular diameters of randomly selected seminiferous tubules were measured in each tom. Five seminiferous tubules from each testis were averaged for each tom so that a Mean±SD could be produced for each age group. The presence of spermatozoa in the lumen was compared using a Chi-square test. Seminiferous tubule diameter was compared using a Student's t test.

An additional parameter of analysis performed in this study group was spermatogenesis and Leydig density scoring of the histopathology slides, completed by a research collaborator (Dr. Kristin Patton). Spermatogenesis scores were quantified using a modified version of the standard Yoshida scoring method (Table II.1). Leydig density was scored on a 0–3 scale: 0=no cells present; 1=scattered/few cells present; 2=moderate number of cells present; 3=densely packed within the interstitium. Spermatogenesis and Leydig cell scoring was compared amongst age groups using a Student's t test. A select group of toms (weanling n=3, juvenile n=4) from this study were further analyzed to see if a relationship existed between Leydig cell density and the presence of penile spines, both indicators of testosterone. The relationship between Leydig cell density and the presence of penile spines was analyzed via linear regression. Significance was defined as $p<0.05$.

Score	Criteria
12	Many late spermatids or spermatozoa (10) present
11	Only a few late spermatids or spermatozoa (<10) present
10	No spermatozoa and no late spermatids, but many round spermatids (10) present
9	No spermatozoa and no late spermatids, but only a few round spermatids (<10) are present
8	No spermatozoa and no spermatids, but many secondary spermatocytes (10) are present
7	No spermatozoa and no spermatids, but only a few secondary spermatocytes (<10) are present
6	No spermatozoa, no spermatids, no secondary spermatocytes, but many primary spermatocytes (10) are present
5	No spermatozoa, no spermatids, no secondary spermatocytes, but only a few primary spermatocytes (<10) are present
4	No spermatozoa, no spermatids, no spermatocytes, many spermatogonia (>10) are present
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

Table II.1. The criteria used for scoring changes within the seminiferous epithelium of FRU toms and kittens in each age group, adapted from Yoshida et al (Yoshida A, Miura K, and Shirai. 1997. Evaluation of seminiferous tubule scores obtained through testicular biopsy examinations of nonobstructive azoospermic men. Fertil Steril 68 (3):514–518.)

Age (months)	Origin	Sample Size
2	FRU	2
2	Pet	2
2.5	FRU	3
2.5	Pet	3
3	FRU	3
3.5	FRU	3
4	FRU	2
4	Pet	2
5	FRU	2
6	FRU	2
Total		**24**

Table II.2. The age (in months), origin, and sample size of FRU and pet queens in the study.

II.B. FRU and Pet Queen Cats

Queens were presented for ovariohysterectomy at a local Corvallis Humane Society in August and September 2015. Based upon previous observations in toms, queens were grouped by age (under 16 weeks (pet n=5, FRU n=10) and 16-24 weeks (pet n=2, FRU n=7)) (Table II.2). Age and life history data from cat colony managers were combined with dental eruption to accurately estimate the age for FRU queens.

A routine sterile ovariohysterectomy was performed under general anesthesia, and all queens recovered from surgery uneventfully. The combined ovarian uterine weights from FRU queens were recorded. Both ovaries from all cats were hemi-sectioned, formalin-fixed, paraffin-embedded, cut into sections (6 μm), and stained with hematoxylin and eosin.

Slides were analyzed using bright field microscopy at 200X by a single observer (EB), blinded to the individual's age group and living status. Follicles were counted and classified as primary, secondary, or tertiary according to Araki (2003). Largest follicle diameter (in μm) was also noted and averaged for all groups. Mean±SD percentages for each classification were compared using a Student's t test. Combined ovarian uterine weights were analyzed via linear regression. Significance was defined as $p<0.05$.

CHAPTER III. RESULTS

III.A. FRU Tom Cats

The presence of penile spines was scored on a numerical scale, with a score of zero being defined as not present, and a score of one representing fully developed penile spines. Eight out of the thirteen juvenile toms had penile spines compared to twelve out of the sixteen adult toms (62% and 75%, respectively). However, these results were not statistically different (p= 0.4539). A number of toms had severe oligozoospermia, which was defined as displaying fewer than fifty sperm on the entire slide (Figure III.1). Three out of the thirteen juvenile toms (23%) in the study demonstrated severe oligozoospermia, as compared to seven out of the sixteen adult toms (44%). These individuals were not included in the overall morphology evaluation. The mean and standard deviation for the percentage of normal sperm morphology for the remaining eleven juvenile toms was 77 ± 11%. Sperm morphology percentages for the remaining nine adult tom cats was 81 ± 13%, respectively. These findings were not significantly different (p=0.5671) (Figure III.1).

The average seminiferous tubule diameter and standard deviation for weanlings, juveniles, pubertal, and adult FRU toms were as follows: 88.10 ± 10.88 µm, 109.8 ± 8.89 µm, 142.2 ± 16.89 µm, 237.90 ± 52.45 µm ($p < 0.05$ between successive age groups) (Figure III.2). The average seminiferous tubular diameter was significantly larger in each successive age group, however the largest increase in diameter between the age groups was observed between pubertal and adult toms, with a 67% difference in average diameter between the two groups. Evidence of spermatogenesis in the weanling, juvenile, pubertal, and adult toms was 0%, 17%, 67%, and 100%, respectively ($p < 0.05$ between

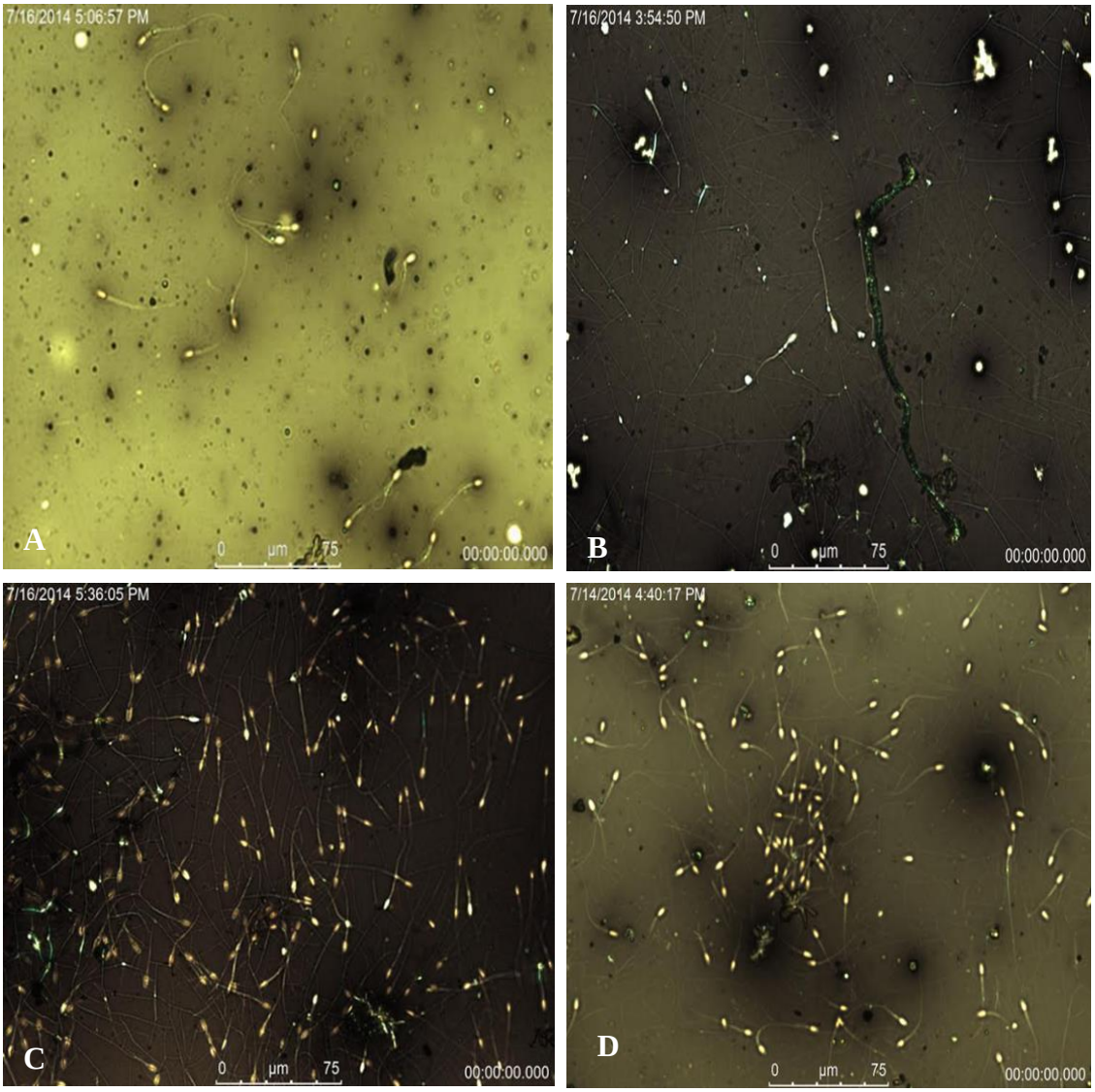

Figure III.1. Oligozoospermic vas deferens secretions in juvenile (A) and adult (B) FRU toms in our study. Vas deferens secretions in juvenile (B) and adult (D) FRU toms in our study.

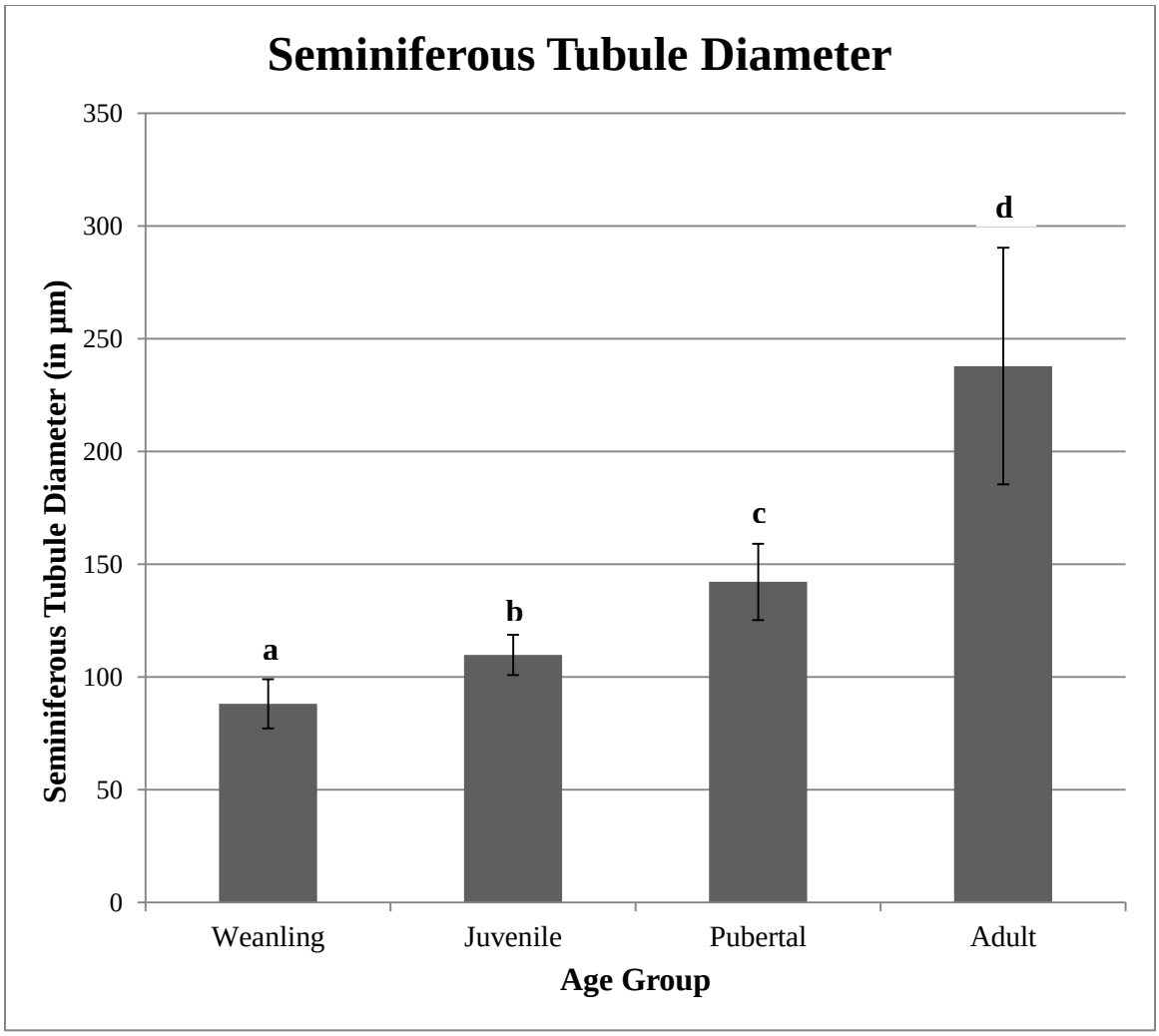

Figure III.2. Graph showing the average seminiferous tubule diameter (in µm) of the various age categories of FRU toms in the study. Groups with different superscripts were significantly different from each other ($p<0.05$).

successive age groups) (Figure III.3). With increasing age group, the presence of spermatozoa observed in the lumen also increased (Figure III.4). The Yoshida score and Leydig cell density for each age group of toms is shown in Table III.1. The Yoshida scores were not significantly different between the weanling and juvenile toms ($p>0.05$). However, there was a significant difference in score between the juvenile and pubertal toms ($p=0.02$). Average seminiferous tubule score in the adult toms was significantly higher than in the weanling, juvenile, and pubertal cats ($p\leq0.01$). Leydig cell density in the weanling toms was significantly higher than that of juvenile, pubertal, and adult toms ($p<0.05$). However, there was a weak correlation observed between Leydig cell density and the presence of penile spines ($r^2=0.53$) (Table III.2).

III.B. FRU and Pet Queens

Representative pictures of gonadal cross-sections of the FRU and pet queens in the study are shown in Figure III.5. A complete representation of pre-ovulatory follicle percentages for each category and age group are demonstrated numerically in Table III.3. FRU cats under 4 months of age had significantly more tertiary follicles compared to pet cats under 4 months of age (33% and 17%, respectively; $p=0.01$). No significant differences were observed between the two age groups of FRU queens and the two age groups of pet queens. The largest average follicle size was observed in the less than 4 month old FRU queens (581.6 ± 53.65 µm). This finding was significantly larger than the follicle diameter size observed in both pet queens at less than 4 months of age and pet queens at 4-6 months of age (502.0 ± 74.51 µm and 469.4 ± 121.48 µm, respectively; $p=0.01$; Figure III.6). It was observed that in FRU cats, uterine weight positively

correlated with age (r^2 = .201; Figure III.7). As evidence of the ovarian follicle endocrine function, combined ovarian uterine weights were significantly higher in 4-6 month (1.18 ± 0.31 g) old FRU queens compared to under 4 month old FRU queens (0.93 ± 0.28 g).

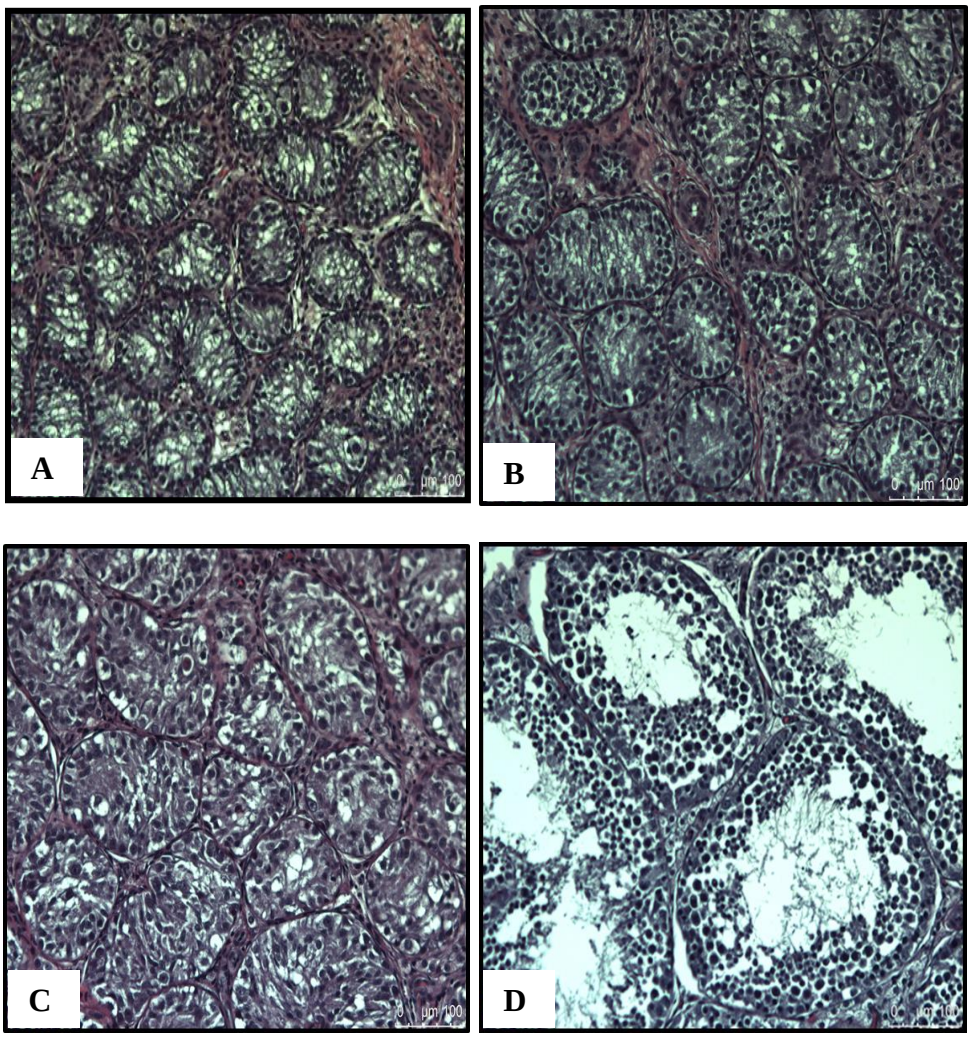

Figure III.3. Slides demonstrating the seminiferous tubule characteristics of weanling (A; 2-2.5 months of age), juvenile (B; 4-5 months of age), pubertal (C; 5-6 months of age), and adult FRU toms in the study (D; 12-24 months of age).

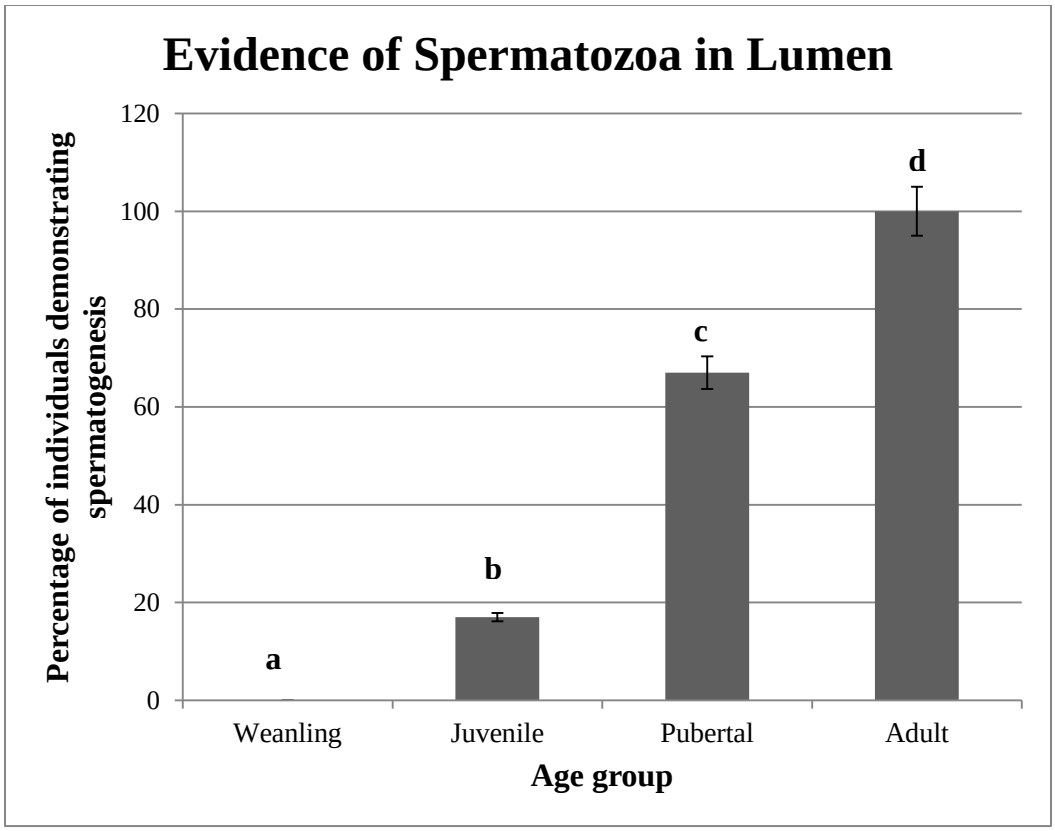

Figure III.4. Graph showing the percentage of individuals demonstrating spermatogenesis (in µm) of the various age categories of FRU toms in the study. Groups with different superscripts were significantly different from each other ($p<0.05$).

Age Category	Spermatogenesis Score	Leydig Density
Weanling (2-2.5 mo)	5.0±2.19	1.83±0.41*
Juvenile (3-4 mo)	4.83±2.04	1.33±0.52
Pubertal (5-6 mo)	8.50±3.15	1.17±0.41
Adult (12-24 mo)	12.0±0.00*	1.0±0.0

Table III.1. Average ± standard deviation for seminiferous tubule score and Leydig density using the Yoshida scoring method.

Age of Tom	Leydig Cell Density	Penile Spine Score
Weanling (2 months)	2	1
Weanling (2 months)	2	1
Weanling (2 months)	2	1
Juvenile (3 months)	1	0
Juvenile (3 months)	2	0
Juvenile (3 months)	2	1
Juvenile (3.5 months)	1	0

Table III.2. Comparison of Leydig cell density and penile spine score in a select group of weanling and juvenile toms.

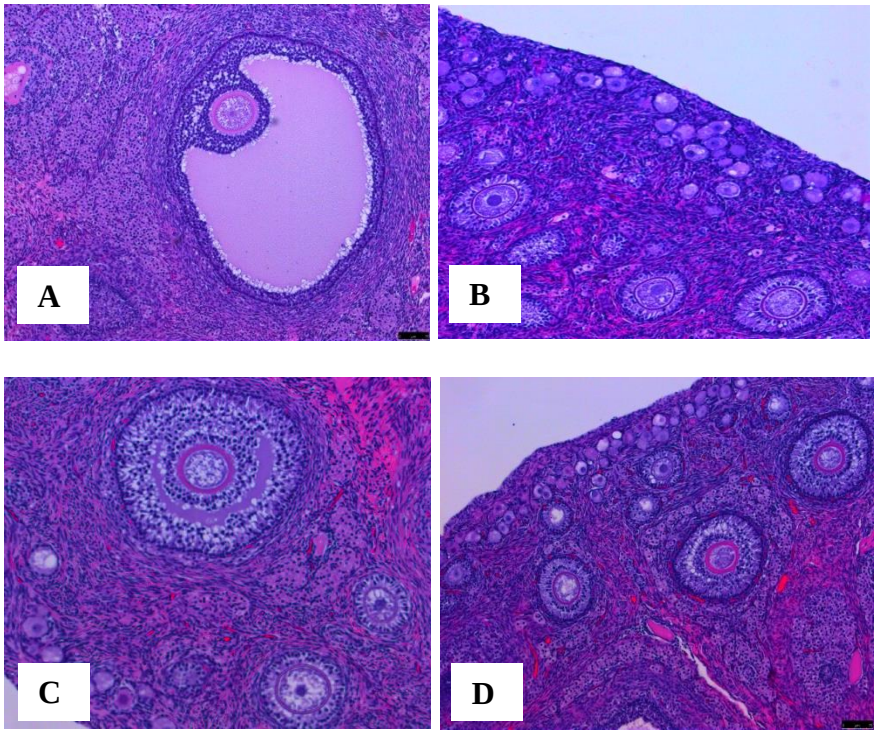

Figure III.5. Representative images of gonadal cross sections from <4 month old FRU queen (A), 4-6 month old FRU queen (B), < 4 month old pet queen (C), and 4-6 month old pet queen (D).

	% Primary	% Secondary	% Tertiary
FRU < 4 months	2.43±12.4	1.29±7.3	3.17±12.9
FRU > 4 months	1.29±17.1	0.69±5.0	1.20±18.1
Pet < 4 months	2.90±4.3	1.70±4.7	1.21±7.6
Pet > 4 months	1.98±25.3	1.11±1.8	2.78±27.1

Table III.3. Mean ± standard deviation percentage of pre-ovulatory follicles in FRU and pet queens.

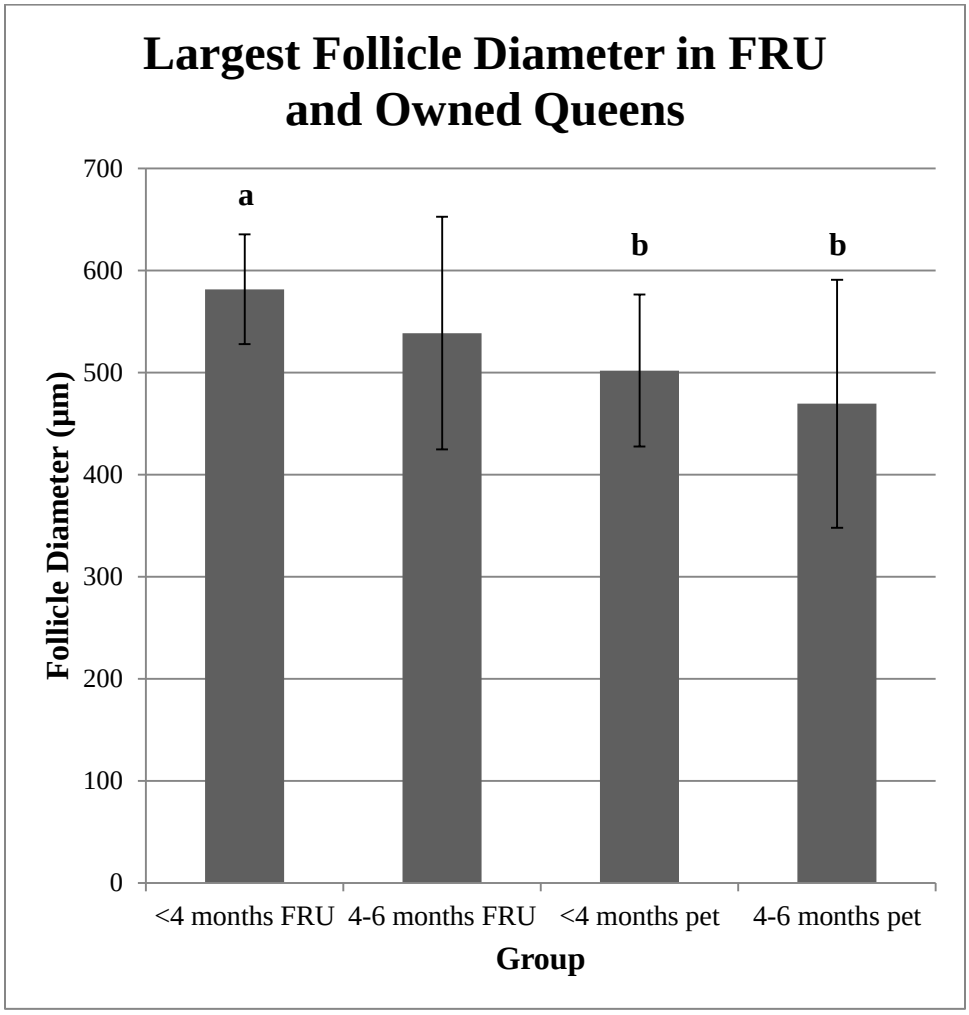

Figure III.6. Average ± standard deviation largest follicle diameter observed in each FRU and pet queen group. Groups with different superscripts were significantly different from each other ($p<0.05$).

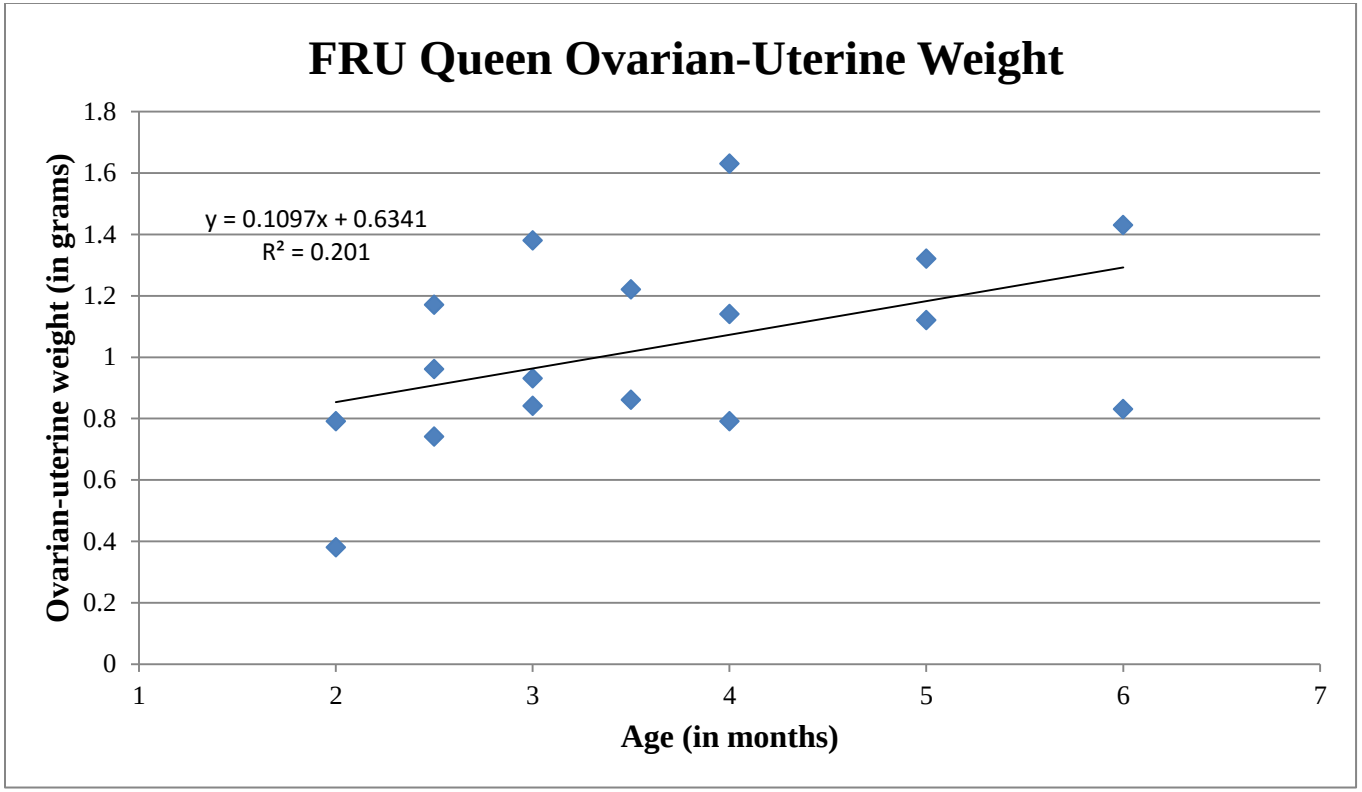

Figure III.7. Combined ovarian-uterine weight of FRU cats at various ages.

CHAPTER IV. DISCUSSION

IV.A. FRU Tom Cats

Ives and coworkers described the eruption of penile spines between 9-13 weeks of age (Ives et al, 1975). In a study performed by Aronson and Cooper, it was observed that in pet cats penile spines increased in size as androgen levels increased, and decreased in size as androgen levels fell. These changes correlated positively with the rise and depression of mating activity, respectively (Aronson and Cooper, 1967). Therefore, toms that do not demonstrate penile spines are less likely to copulate and contribute less to the population. It is not clear why more of the juvenile toms in the study did not have penile spines present at the time of castration. Additionally, it is unclear why only 75% of the adult toms in the study demonstrated fully-developed penile spines. These findings may have been related to the over- and under-estimation of the age of the animals. The toms in our study were aged via dental eruption patterns. Age determination via dentition is less accurate in animals with brachyodont incisors (i.e. cats, dogs, and cattle) than in hypsodont dentition (i.e. equines) (Merck et al, 2015). Therefore, dental eruption patterns are not a completely accurate estimate of age in cats. Inaccurate age estimates may have played a large role in the results found in this particular group of FRU toms. Severe oligospermia in several of the toms (23% of the juvenile toms and 44% of the adult toms) was an unexpected finding given the overall high observed fertility of this population. However, we did find some evidence to suggest that juvenile and adult normospermic FRU cats had higher amounts of normal sperm morphology (77±11% and

81±13%, respectively), one possible indicator of fertility, than what has been reported for domestic cats (~70%) (Howard et al, 1990).

Juvenile (3-4 months of age) FRU toms in the current study had significantly wider tubular diameter (109.8 ± 8.89 μm) than what has been previously reported for domestic toms under 5 months of age (86 μm) (Sanchez et al, 1993b). This supports our hypothesis that spermatogenesis is occurring at an earlier age in FRU toms than what has been previously reported in pet domestic toms (8-10 months). However, the time of the year in which the measurements were taken in the Sanchez study was not reported. If these measurements were made outside of the breeding season, this may explain why the tubular diameters were smaller than the diameters that were found in the current study. There were some conflicting results amongst the two studies investigating the presence of spermatogenesis in FRU toms. Evidence of spermatogenesis in the weanling, juvenile, pubertal, and adult toms was 0%, 17%, 67%, and 100%, respectively, on the basis of presence of spermatozoa in the lumen. However, when analyzed using the Yoshida system, seminiferous tubule scores were very poor amongst weanling, juvenile, and pubertal cats. The only group that demonstrated a Yoshida score that reflected the presence of late spermatids or spermatozoa were the adult (12-24 month old) FRU toms. This finding does not support our hypothesis that FRU toms are reaching puberty sooner than what has been previously reported for pet domestic toms. This finding was also surprising given that seminiferous tubule diameter, one indicator of spermatogenesis, appeared to be higher amongst juvenile FRU cats than what had previously been reported for domestic cats at the same age. The weanling toms in the current study had the highest Leydig cell density score. These results are supported by previous work in pet toms with

the number of Leydig cells decreasing progressively from 1 to 4 months of age, and by 3-4 months of age only a small number of cells are present (Sanchez et al, 1993b).

There may be a connection between the living status of FRU cats and the age at which they reach reproductive capacity. In study one, the group of feral toms analyzed for the presence of penile spines and sperm morphology on average demonstrated remarkably high levels of normal spermatozoa, even under 6 months of age. Conversely, in the group of FRU individuals analyzed for spermatogenesis, all of the toms under 6 months of age demonstrated low Yoshida scores. The first group was composed solely of feral toms, whereas the second group was composed mostly of community-owned cats. As feral cats are self-sustaining and do not have their food and water provided by humans, they may not live as long as community-owned cats, who have their food and water consistently provided by humans. Because feral cats are not provided food, they may die sooner than community-owned cats. Due to this selective pressure, feral cats may be forced to reproduce even sooner than their community-owned counterparts.

IV.B. FRU and Pet Queens

In support of our hypothesis that FRU queens are reaching reproductive capacity sooner than pet domestic cats, FRU cats under 4 months of age had significantly more tertiary follicles compared to pet cats under 4 months of age (33% and 17%, respectively). As tertiary follicles are the class of follicle that is subsequently ovulated, this finding demonstrates that FRU queens have the capability to ovulate at ages under 4 months, which is significantly sooner than what has been previously reported for pet domestic queens (8-10 months of age).

The largest average follicle size was observed in the less than 4 month old FRU queens. This finding was significantly larger than the follicle diameter size observed in both pet queens at less than 4 months of age and pet queens at 4-6 months of age. As follicle diameter size has been positively correlated with reproductive capacity, these results show evidence to support that FRU queens are reaching reproductive capacity at an earlier age. It is important to note that the average tertiary follicle diameter measurements in the <4 month old cats in the current study were found to be significantly larger (581.6 ± 53.65 μm) than what has been previously reported in queens by Reynaud et al (223.8 μm in queens aged on average to be 1.17 years old, living status unknown) (Reynaud et al, 2009). However, other studies have found tertiary follicle diameters to range anywhere from 300-1000 μm (Bristol-Gould and Woodruff, 2006). Studies have demonstrated that dehydration and paraffin embedding are known to induce 10-15% of tissue retraction (Iwadare et al, 1984). The smaller reported average follicle sizes in the Reynaud study and the current study may be attributed to this process.

A significant increase in ovarian-uterine weight was observed in FRU queens 4-6 months of age. In the Uchikura study, it appeared that the increase in ovarian weight observed in pre-pubertal FRU queens could be largely contributed to the increase in the number and size of tertiary follicles (Uchikura et al, 2010). However, in the current study we found no difference in both the tertiary follicle number and size between <4 month old queens and 4-6 month old queens. Therefore, other factors causing increased ovarian-uterine weight may be at play. Latimer et al (1939) found the average ovarian-uterine weight in domestic, non-pregnant felines to be 1.5 grams. This is slightly higher than the weights observed in both the 4-6 month old FRU queens (1.18 ± 0.31 g) and the under 4

month old FRU queens (0.93 ± 0.28 g) in our study. However, in our data the r^2 value was low, attributed to a high amount of scatter amongst the two groups of FRU queens. There was a considerable amount of variation in the body condition of the queens of various ages observed in our study. Body condition is a fairly representative indicator of the overall health of the animal. As this was an observational study, food, water, and shelter resources were not consistent or controlled amongst all FRU cats. Therefore, much of the variation in our study (in both FRU toms and queens) can probably be contributed to the variation in body condition and overall health observed in the FRU cats.

CHAPTER V. CONCLUSIONS AND FUTURE DIRECTIONS

Our studies demonstrate some evidence to suggest that FRU cats are reaching reproductive capacity at an earlier age than what has been previously reported for pet domestic cats. Interestingly, the different sexes of FRU cats do not seem to be reaching reproductive capacity at the same age. Based on the results found in our study, FRU queens appear to be reaching reproductive capacity before 4 months of age (increased number and size of tertiary follicles). FRU toms do not appear to be reaching reproductive capacity at a consistent age. However, there is some evidence to suggest that FRU toms are reaching reproductive capacity at an earlier age than pet domestic cats (high levels of morphologically normal sperm, larger seminiferous tubule diameters, and increased Leydig cell density in toms less than 6 months of age).

Selective pressures including a severely shortened life span may be factors contributing to these findings. The average life span of FRU cats is 2 years of age, with as many of 90% of kittens dying before 6 months of age in some locations (van Aarde, 1984). FRU cats may have adapted to this pressure by collectively decreasing the age at which they can reproduce, ensuring the propagation of their genetic material before their untimely death.

Our findings are important to the veterinary and population control community. Our data suggests that sterilization efforts should be focused on FRU kittens, especially females under 4 months of age. Our discoveries may not only help to ease the suffering of millions of animals, but also help to lower the transmission of dangerous diseases between FRU and pet cats, and restore balance amongst ecological communities.

Additional studies using FRU cats from other geographical areas are needed to determine if this observation is an isolated phenomenon or worldwide in distribution. Further investigation into the hormones involved in sexual maturity and reproduction (i.e. testosterone, estrogen, luteinizing hormone, etc.) in juvenile and adult FRU cats may provide additional evidence to support our findings. Studies investigating if a reproductive difference exists between feral and community-pet cats may also help population control programs target specific populations that demonstrate the highest overall fertility.

Our results suggest that FRU females under 4 months of age appear to be a target group for population control. Current protocols dictate that kittens must be at least two months old to undergo anesthesia and subsequent surgical sterilization. Research into effective non-surgical sterilization that can be administered to young kittens may be highly beneficial in helping to control the FRU population. More research focused on creating effective, long-lasting sterilization vaccines is needed.

CHAPTER VI. REFERENCES

AVMA (American Veterinary Medical Association). 2012. U.S. Pet Ownership & Demographic Sourcebook. Accessed on January 12, 2016 from: https://www.avma.org/KB/Resources/Statistics/Pages/Market-research-statistics-US-Pet-Ownership-Demographics-Sourcebook.aspx .

Araki Y. 2003. Formation and structure of mammalian ovaries. In Tulsiani D (ed), Introduction to Mammalian Reproduction. Boston: Kluwer Academic Publishers, pp. 141-155.

Aronson LR, Cooper ML. 1967. Penile spines of the domestic cat: their endocrine-behavior relations. Anat Rec 157:71-78.

Axner E, Linde Forsberg C. 2007. Sperm morphology in the domestic cat, and its relation with fertility: a retrospective study. Reprod Domest Anim 42:282-291.

Batson A. 2008. Global companion animal ownership and trade: project summary, June 2008. World Society for the Protection of Animals. Accessed January 12, 2016, from: http://s3.amazonaws.com/zanran_storage/www.wspa.org.uk/ContentPages/48536804.pdf

Berndtson WE. 1977. Methods for quantifying mammalian spermatogenesis: a review. J Anim Sci 44:818-833.

Blottner W, Jewgenow K. 2007. Moderate seasonality in testis function of domestic cat. Reprod Domest Anim 42:536-540.

Bohrer E, Mihalyo A, Kutzler M. 2015. Histologic and morphometric evaluation of testes of feral tom kittens and cats. Society for Theriogenology Annual Conference Proceedings 2015 (abstract).

Bristol K, Woodruff K. 2004. Follicle-restricted compartmentalization of transforming growth factor beta superfamily ligands in the feline ovary. Biol Reprod 70:846-59.

Bristol-Gould S, Woodruff TK. 2006. Folliculogenesis in the domestic cat (*Felis catus*). Theriogenology 66:5-13.

Christian KA, Blanton JD, Auslander M, Rupprecht CE. 2009. Epidemiology of rabies post-exposure prophylaxis-United States of America, 2006-2008. Vaccine 27: 7156-7161.

Colby E. 1980. Infertility and disease problems. In: Morrow DE (ed): Current Therapy in Theriogenology. Philadelphia, WB Saunders Co, pp. 869.

Coman BJ, Jones EH, Westbury HA. 1981. Protozoan and viral infections of feral cats. Australian Veterinary Journal 57:319-323.

Conrad PA, Miller MA, Kreuder C, James ER, Mazet J, Dabritz H, Jessup DA, Gulland F, Grigg ME. 2005. Transmission of toxoplasma: clues from the study of sea otters as sentinels of *Toxoplasma gondii* flow in the marine environment. Int J Parasitol 35:1155-1168.

Cornell University College of Veterinary Medicine. 2014. Feline Leukemia Virus. Accessed February 3, 2016 from: http://www.vet.cornell.edu/fhc/Health_Information/brochure_felv.cfm .

Courot M, Hochereau-de Reviers MT, Ortavant R. 1970. Spermatogenesis. In: Johnson AD, Gomes WR, Vandemark NL (eds.), The Testis. New York, Academic Press, pp.339-432.

Danner RM, Goltz DM, Hess SC, Banko PC. 2007. Evidence of feline immunodeficiency virus, feline leukemia virus, and *Toxoplasma gondii* in feral cats on Mauna Kea, Hawaii. J Wildl Dis 43:315-318.

Desjardins C, Hafs HD. 1969. Maturation of bovine female genitalia from birth through puberty. J Anim Sci 28:502-507.

Elliot DL, Tolle SW, Goldberg L. 1986. Pet-associated illness. J Am Anim Hosp Assoc 22:387-399.

Foley P, Foley JE, Levy JK, Paik T. 2005. Analysis of the impact of trap-neuter-return programs on populations of feral cats. J Am Vet Med Assoc 227:1775-1781.

Franca LR, Godinho CL. 2003. Testis morphometry, seminiferous epithelium cycle length, and daily sperm production in domestic cats (*Felis catus*). Biol Reprod 68:1554-1561.

Gibson AK, Raverty S, Lambourn DM, Huggins J, Magargal SL, Grigg ME. 2011. Polyparasitism is associated with increased disease severity in *Toxoplasma gondii*-infected marine sentinel species. PLoS Neglected Tropical Diseases 5:e1142. doi: 10.1371.

Goodrowe KL, Howard JG, Schmidt PM, Wildt DE. 1989. Reproductive biology of the domestic cat with reference to endocrinology, sperm function, and in-vitro fertilization. J Reprod Fertil Suppl 39:73-90.

Gosling L, Stavinsky J, Dean R. 2013. What is a feral cat? Variation in definitions may be associated with different management strategies. J Feline Med Surg 15:759-764.

Grimm D. 2009. A cure for euthanasia? Science 325:1490-1493.

Gudermuth D, Newton L, Daels P, Concannon P. 1997. Incidence of spontaneous ovulation in young, group-housed cats based on serum and faecal concentrations of progesterone. J Reprod Fertil Suppl 51:177-184.

Hawkins C, Grant W, Longnecker M. 2004. Proceedings of the 4th International Urban Wildlife Symposium (eds: Shaw WW, Harris LK, Vandruff L), University of Arizona, Tucson, AZ, pp. 164-170.

Hensley JA. 1998. Potential rabies exposures in a Virginia county. Public Health Report 113:258-262. Accessed on January 22, 2016 from: http://www.ncbi.nlm.nih.gov/pubmed/9633873.

Howard JG, Brown JL, Bush M, Wildt DE. 1990. Teratospermic and normospermic domestic cats: ejaculate traits, pituitary-gonadal hormones, and improvement of spermatozoal motility and morphology after swim-up processing. J Androl 11:204-215.

Howard JG, Donoghue AM, Johnston LA, Wildt DE. 1993. Zona pellucida filtration of structurally abnormal spermatozoa and reduced fertilization in teratospermic cats. Biol Reprod 49:131-139.

Hughes KL, Slater MR, Haller L. 2002. The effects of implementing a feral cat spay/neuter program in a Florida county animal control service. J Appl Anim Welf Sci 4:285-298.

Humane Society of the United States. N.d. Pets by the numbers; pet ownership, community cat and shelter population estimates. Accessed on February 1, 2016 from: http://www.humanesociety.org/issues/pet_overpopulation/facts/pet_ownership_statistics.html?referrer=https://www.google.com/

Humphrey S, Barbour D. 1981.Status and habitat of three subspecies of *Peromyscus polionotus* in Florida. Journal of Mammology 62:840-844.

Hurni H. 1981. Daylength and breeding in the domestic cat. Lab Anim 15:229-233.

Ives PJ, McArthur NH, Sis FR. 1975. Scanning electron microscopy of the penile spines of the domestic cat. Southwestern Veterinarian 28:58-59.

Iwadare T, Mori H, Ishiguro K, Takeishi M. 1984. Dimensional changes of tissues in the course of processing. J Microsc 136:323-327.

Jemmett JE, Evans JM. 1977. A survey of sexual behavior and reproduction of female cats. J Small Anim Pract 18:31-37.

Joffre M. 1977. Relationships between testicular blood flow, testosterone secretion and spermatogenic activity in young and adult red foxes (*Vulpes vulpes*). J Reprod Fertil 51:35-40.

Johnston SD, Root-Kustritz MV, Olson PNS. 2001. Canine and Feline Theriogenology. St. Louis, Elsevier Health Sciences.

Kirkpatrick J. 1985. Seasonal testosterone levels, testosterone clearance, and testicular weights in male domestic cats. Canadian Journal of Zoology 63:1285-1287.

Lappin M. 1993. Feline zoonotic diseases. Vet Clin North Am Small Anim Pract 23:57-78.

Latimer HB. 1939. The prenatal growth of the cat. VIII. The weights of the kidneys, bladder, gonads, and uterus, with the weights of the adult organs. Growth 3:89-108.

Lee IT, Levy JK, Gorman SP, Crawford PC, Slater MR. 2002. Prevalence of feline leukemia virus infection and serum antibodies against feline immunodeficiency virus in unowned free-roaming cats. J Am Vet Med Assoc 220:620-622.

Leme D, Papa F, Trinca L. 2002. Assessment of seasonal influence of equine spermatogenesis cells and Sertoli cells by testicular fine needle aspiration cytology. Theriogenology 58:269-272.

Levy JK, Gale DW, Gale LA. 2003. Evaluation of the effect of a long-term trap-neuter-return and adoption program on a free-roaming cat population. J Am Vet Med Assoc 222:42-46.

Levya H, Madley T, Stabenfeldt GH. 1989. Effect of light manipulation on ovarian activity and melatonin and prolactin secretion in the domestic cat. J Reprod Fertil Suppl 39:125-133.

Loss S, Will T, Marra P. 2013. The impact of free-ranging domestic cats on wildlife in the United States. Nature Communications 4:1396. doi:10.1038/ncomms2380. Accessed on

January 4, 2015 from: http://www.nature.com/ncomms/journal/v4/n1/full/ncomms2380.html .

Lowe S, Browne M, Boudjelas S. 2000. 100 of the worst invasive species: a selection from the global invasive species database (Invasive Species Specialist Group, International Union for Conservation of Nature, 2000). Accessed on January 22, 2016, from:http://www.planta.cn/forum/files_planta/100_worst_invasive_species_english_941.pdf

Mahdi D, Kahlilli K. 2008. Relationship between follicle growth and circulating gonadotropin levels during postnatal development of sheep. Anim Reprod Sci 106:100-112.

McCarthy J. 2013. Estimation of effectiveness of three methods of feral cat population control by use of a simulation model. J Am Vet Med Assoc 243:502- 511.

Medina F, Bonnaud E, Vidal E, Terhy BR, Zavaleta ES, Donlan CJ, Keitt BS, Corre ML, Horwath SV, Nogales M. 2011. A global review of the impacts of invasive cats on island endangered vertebrates. Global Change Biol 17:3503-3510. Accessed on January 10, 2016 from: http://advancedconservation.org/library/medina_etal_2011.pdf

Medlock K, Forrester T, Sheehan D. 1994. Progesterone and estradiol interaction in the regulation of rat uterine weight and estrogen receptor concentration. Proc Soc Exp Biol Med (New York) 2:146-53.

Merck, Sharp, and Dohm Corp., a subsidiary of Merck & Co., Inc. 2015. Merck Veterinary Manual. Accessed on January 15, 2016 from: http://www.merckvetmanual.com/mvm/digestive_system/dental_development/estimation_of_age_by_examination_of_the_teeth.html

Mirmovitch V. 1995. Spatial organization of urban feral cats (*Felis catus*) in Jerusalem. Wildlife Reserves 22:290-310.

Moore DA, Sischo WM, Hunter A, Miles T. 2000. Animal bite epidemiology and surveillance for rabies postexposure prophylaxis. J Am Vet Med Assoc 217:190-194.

Natoli E, Maragliano L, Cariola G, Faini A, Bonanni R, Cafazzo S, Fantint C. 2006. Management of feral domestic cats in the urban environment of Rome (Italy). Preventive Veterinary Medicine. 77:180-185.

Neville PF, Remfry J. 1984. Effect of neutering on two groups of feral cats. Vet Rec 114:447-450.

NOAA Fisheries. 2013. Hawaiian monk seals and *Toxoplasma gondii*. Accessed on January 18, 2016, from: http://www.pifsc.noaa.gov/hawaiian_monk_seal/health_and_disease.php

Nutter F, Levine J, Stoskopf M. 2004. Reproductive capacity of free-roaming domestic cats and kitten survival rate. J Am Vet Med Assoc 9:1399-1402.

Nutter F, Stoskopf M. 2004. Time and financial costs of programs for live trapping feral cats. J Am Vet Med Assoc 225:1403-1405.

Patton. 2015. Scorer of the spermatogenesis and Leydig density on the histopathology slides, Theriogenology laboratory, Oregon State University.

Pineda MH, Dooley MP. 1984. Surgical and chemical vasectomy in the cat. Am J Vet Res 45:291-300.

Reif JS. 1980. Toxoplasmosis: assessment of the role of cats in human infection. Compendium on Continuing Education for the Practicing Veterinarian 2:810-814.

Reiter R. 1980. The pineal and its hormones in the control of reproduction in mammals. Endocr Rev 1:109-131.

Rey R. 1999. The prepubertal testis: a quiescent or a silently active organ? Histol Histopathol 14:991-1000.

Reynaud K, Gicquel C, Thoumire S, Chebrout M, Ficheux C, Bestandji M, Chastant-Maillard S. 2009. Folliculogenesis and morphometry of oocyte and follicle growth in the feline ovary. Reprod Domest Anim 22:174-179.

Richards JR, Elston TH, Ford RB, Gaskell RM, Hartmann K, Hurley KF, Lappin MR, Levy JK, Rodan I, Scherk M, Schultz RD, Sparkles AH. 2006. The 2006 American Association of Feline Practitioners feline vaccine advisory panel report. J Am Vet Med Assoc 229:1405-1441.

Robertson S. 2007. A review of feral cat control. J Feline Med Surg 10:366-375.

Root MV, Johnston SD, Olson PMS. 1995. Estrous length, pregnancy rate, gestation and parturition lengths, litter size, and juvenile mortality in the domestic cat. J Am Anim Hosp Assoc 31:429-433.

Sanchez B, Pizarro M, Garcia P, Flores JM. 1993a. Histological study of Leydig cells in the cat from birth to sexual maturity. J Reprod Fertil Suppl 47:349-353.

Sanchez B, Pizzaro M, Garcia P, Flores JM. 1993b. Postnatal development of seminiferous tubules in the cat. J Reprod Fertil Suppl 47:343-348.

Siemieniuch MJ, Woclawek-Potocka I. 2007. Morphological features of the seminiferous epithelium in cat (*Felis catus*) testes. J Reprod Dev 53:1125-30.

Slater MR. 2005. The welfare of feral cats. In: Rochlitz I (ed), The Welfare of Cats. Netherlands, Springer, pp.141-175.

Shille VM, Stabenfeldt GH. 1980. Current concepts in reproduction of the dog and cat. Adv Vet Sci Comp Med 24:211-43.

Tiptanavattana N, Radtanakatikanon A, Buranapraditikun S, Hyttel P, Holmes HM, Setthawong P, Techakumphu M, Thrasanit T. 2014. 94 chronological transition of gonocytes to spermatogonial stem cells during pre-pubertal and pubertal periods in domestic cats. Reproduction, Fertility, and Development 27:140.

Tsutsui T, Stabenfeldt GH. 1993. Biology of ovarian cycles, pregnancy and pseudopregnancy in the domestic cat. J Reprod Fertil 47:29-35.

Tsutsui T, Nakagawa K, Hirano T, Nagakubo K, Shinomiya M, Yamamoto K, Hori T. 2004. Breeding season in female cats acclimated under a natural photoperiod and interval until puberty. J Vet Med Sci 66:1129-1132.

Uchikura K, Nagano M, Hishinuma M. 2010. Evaluation of follicular development and oocyte quality in pre-pubertal cats. Reprod Domest Anim 45:405-411.

van Aarde RJ. 1984. Population of feral cats on Marion Island. Acta Zool Fennica 172: 107-110.

Wildt D, Seager S. 1978. Ovarian response in the estrual cat receiving varying doses of HCG. Horm Res 9:114-150.

Wildt D. 1980. Laparoscopic determination of ovarian and uterine morphology during the reproductive cycle. In: Morrow DE (ed), Current Therapy in Theriogenology. Philadelphia, WB Saunders Co.

Wildt D, Bush M, Howard J, O'Brien S, Meltzer D, Van Dyk A, Ebedes H, Brand D. 1983. Unique seminal quality in the South African cheetah and a comparative evaluation in the domestic cat. Biol Reprod 29:1019-1025.

Yoshida A, Miura K, and Shirai. 1997. Evaluation of seminiferous tubule scores obtained through testicular biopsy examinations of nonobstructive azoospermic men. Fertil Steril 68:514–518.

APPENDIX A. ABSTRACT ACCEPTED FOR POSTER PRESENTATION AT THE 2014 NORTHWEST REPRODUCTIVE SCIENCES SYMPOSIUM IN CLE ELUM, WA

Comparison of penile spines and sperm morphology between juvenile and adult feral cats

Ellie Bohrer, Anna Mihalyo, Michelle Kutzler
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Background: Hundreds of thousands of dollars and volunteer hours are spent on trapping, neutering, and releasing feral cats with no apparent effect on the size of the population.[1] Therefore, we hypothesized that feral tom cats have reproductively adapted to man-made sterilization efforts by lowering the age at which they enter puberty and by increasing their fertility as compared to domestic cats based upon published normal ranges.[2] The objective of this study was to compare the presence of penile spines and the percentage of normal spermatozoa expressed from the vasa deferentes of juvenile and adult feral tom cats.

Methods: Tom cats were presented for castration at a feral cat neutering clinic. The age ranges were: 2-6 months (juvenile; n=13) and over 6 months (adult; n=16) Age was estimated on the basis of dental eruption. After inducing general anesthesia, the penis was evaluated to determine if spines were present. Next, a routine open castration was performed. Contents from both vasa deferentes were milked onto a microscope slide, mixed with eosin-nigrosin stain, spread with a spreader slide, and allowed to air dry. The smears were blindly evaluated by the same observer (EB) using bright field microscopy under oil immersion (1000X) and the percentage of sperm with normal morphology was determined after evaluating 100 sperm per slide. The presence of penile spines was compared using a Chi-Square test and the percent morphologically normal sperm was compared using a Student's t test; where $p<0.05$ was defined as significant.

Results: More adult toms (16/16) than juvenile toms (4/13) had penile spines ($p<0.05$). Several toms (8/13 juveniles and 6/16 adults) displayed fewer than 50 sperm on the slide. The results from these smears were therefore not included in the evaluation of sperm morphology. The mean±SD percentage of morphologically normal sperm for the remaining five juvenile and ten adult tom cats was not significantly different (77±11% and 81±13%, respectively).

Discussion: Ives et al. described the eruption of penile spines to occur between 9-13 weeks of age.[3] It is not clear why more of the juvenile toms did not have penile spines present at the time of castration, but this may have been related to over-estimation of the age of the animals in our study. Based on our findings we conclude that feral tom cats in this population do not reach puberty any earlier than toms in populations not subjected to population control measures, however we did find some evidence that the feral cats had better sperm morphology, one possible indicator of fertility, than has been reported for domestic cats (~70%)[3]. Further studies, comparing toms of known ages and both populations (domestic vs. feral) will have to be conducted to better test our hypothesis.

Keywords: Feline, Neuter, Oligospermia, Puberty, Vas Deferens

References: [1]Grimm D. A cure for euthanasia? Science 2009;325:1490-3.
[2]Howard JG, Brown JL, Bush M, et al. Teratospermic and normospermic domestic cats: ejaculate traits, pituitary-gonadal hormones, and improvement of spermatozoal motility and morphology after swim-up processing. Journal of Andrology 1990;11:204-15.
[3]Ives PJ, McArthur NH, Sis FR. Scanning electron microscopy of the penile spines of the domestic cat. Southwestern Veterinarian 1975;28:58-9.

APPENDIX B. 2014 ABSTRACT PRESENTED AT THE 2014 SOCIETY FOR THERIOGENOLOGY CONFERENCE IN PORTLAND, OR

Comparison of penile spines and sperm morphology in juvenile and adult feral tom cats

Ellie Bohrer, Anna Mihalyo, Michelle Kutzler
Department of Animal and Rangeland Sciences, Oregon State University

Background: In the United States, the feral cat overpopulation crisis continues to persist seemingly unchecked. Hundreds of thousands of dollars and volunteer hours are spent on trapping, neutering, and releasing feral cats with no apparent dent in the population being made.[1] Therefore, we hypothesized that feral tom cats have reproductively adapted to man-made sterilization efforts by lowering the age at which they enter puberty and increasing their fertility. The objective of this study was to compare the presence of penile spines and the percentage of normal spermatozoa expressed from the vas deferens in juvenile and adult feral tom cats.

Methods: Tom cats were presented for castration at a feral cat neutering clinic. The age ranges were: 2-6 months (juvenile; n=13) and over 6 months (adult; n=16). After inducing general anesthesia, the penis was evaluated to determine if spines were present. Next, the scrotal hair was clipped, the skin was aseptically prepped for surgery, and a routine open castration was performed. Contents from both vas deferens were milked onto a microscope slide, mixed with eosin-nigrosin morphology stain, spread with a spreader slide, and allowed to air dry. The slides were blindly evaluated by the same observer (EB) using bright field microscopy under oil immersion (1000X) and the percentage of sperm with normal morphology was determined. The presence of penile spines was compared using a Chi-Square test and the percent normal morphology was compared using a Student's t test; where $p<0.05$ was defined as significant.

Results: More penile spines were present in adult toms (12/16) compared to juvenile toms (8/13) ($p<0.05$). Several toms (3/13 juveniles and 7/16 adults) had severe oligospermia (defined as fewer than 50 sperm present on the entire slide) and were not included in the morphology evaluation. The mean±SD percentage normal sperm morphology for the remaining five juvenile and ten adult tom cats was not significantly different (77±11% and 81±13%, respectively).

Discussion: Ives and coworkers described the eruption of penile spines between 9-13 weeks of age.[2] It is not clear why more of the juvenile toms did not have penile spines present at the time of castration, but this may have been related to over-estimation of the age of the animals. Severe oligospermia was an unexpected finding given the overall high fertility of this population. However, the toms without severe oligospermia had normal sperm morphology percentages that were higher than what has been previously reported in domestic cats (~70%).[3]

Keywords: Feline, Neuter, Oligospermia, Puberty, Vas Deferens

References:

[1] Grimm D. A cure for euthanasia? Science 2009;325:1490-3.

[2]Ives PJ, McArthur NH, Sis FR. Scanning electron microscopy of the penile spines of the domestic cat. Southwestern Veterinarian 1975;28:58-9.

[3]Howard JG, Brown JL, Bush M, et al. Teratospermic and normospermic domestic cats: ejaculate traits, pituitary-gonadal hormones, and improvement of spermatozoal motility and morphology after swim-up processing. Journal of Andrology 1990;11:204-15.

APPENDIX C. POSTER PRESENTED AT THE 2015 CELEBRATION OF UNDERGRADUATE EXCELLENCE CORVALLIS, OR

Histologic and Morphometric Evaluation of Testes of Feral Tom Kittens and Cats

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Oregon State University, Corvallis, Oregon

INTRODUCTION

- Even with the introduction of Trap-Neuter-Release programs, the world's feral cat population continues to remain seemingly uncontrollable[1].
- We are interested in discovering the biological reasons why feral cats are so reproductively successful.
- Previous work by our laboratory has shown that normal morphologic sperm are present in vas deferens secretions of feral toms before 6 months of age.[2]

OBJECTIVES & HYPOTHESIS

- We hypothesize that the onset of spermatogenesis occurs before 6 months of age in feral toms.
- The study objective was to histologically evaluate testes from weanling (2 months of age) through adulthood (24 months of age) to determine when the onset of spermatogenesis occurs in feral toms.

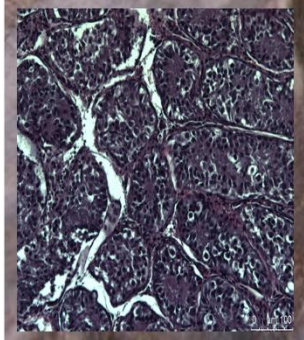

Figure 1. Histopathology of seminiferous tubules in a pubertal feral tom cat.

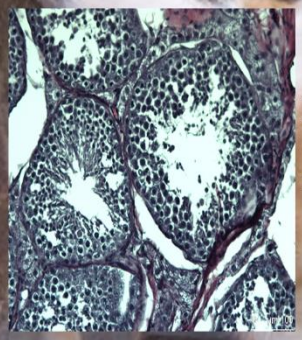

Figure 2. Histopathology of seminiferous tubules in an adult feral tom cat.

MATERIALS & METHODS

- Feral toms were presented for castration at a local Humane Society during August-October 2014.
- Age was determined by records provided from feral cat colony managers and confirmed with dental eruption patterns.
- The age groups were: 2-2.5 months (weanling; n=6), 3-4 months (juvenile; n=6), 5-6 months (pubertal; n=6), and 12-24 months (adult; n=6).
- General anesthesia was induced and a routine open castration was performed. Both testicles from each cat were hemi-sectioned, formalin-fixed, paraffin-embedded, cut into sections (6 µm), and stained with hematoxylin and eosin.
- The slides were evaluated by a single observer (EB) blinded to age group using bright field microscopy (200X).
- Evidence of spermatogenesis was determined on the basis of presence of spermatozoa in the seminiferous tubule lumen.
- In addition, perpendicular diameters measured from 5 tubules for each testis were averaged and mean ± SD was determined for each age group.
- Tubular diameter was compared using a Student's t test where $p < 0.05$ was defined as significant.
- The presence of spermatozoa in the lumen was compared using a Chi -square test.

RESULTS

- Evidence of spermatogenesis in weanling, juvenile, pubertal, and adult toms was 0%, 17%, 67%, and 100%, respectively ($p < 0.05$ between successive age groups).
- The seminiferous tubular diameter was significantly larger in each successive age group (weanlings 88.10 ± 10.88 µm; juveniles 109.8 ± 8.89 µm; pubertal 142.2 ± 16.89 µm; adult 237.90 ± 52.45 µm).

DISCUSSION

- Juvenile feral toms in the current study had significantly wider tubular diameter than previously reported for domestic toms under 5 months of age (86 µm).[3]
  - This supports our hypothesis that spermatogenesis is occurring at an earlier age in feral toms.
- However, the time of year the previous measurements in domestics was not reported.
  - If these measurements were made outside of the breeding season, this may explain why the tubular diameters were smaller.
  - Future studies are planned to determine if folliculogenesis occurs earlier in queens as well.

ACKNOWLEDGEMENTS

Special thanks to Heartland Humane Society for their help and support, and the Metaphorical Platypus website for the background photograph.

LITERATURE CITED

1. Tiptanavittana N., et al. Reprod Fert Dev 2014; 27(1):140.

2. Bohrer E., et al. Comparison of penile spines and sperm morphology between juvenile and adult feral cats. Society for Theriogenology Annual Conference Proceedings 2014 (abstract).

3. Sanchez B., et al. J Reprod Fert Suppl 1993; 47:343.

APPENDIX D. ABSTRACT PRESENTED AT THE 2015 SOCIETY FOR THERIOGENOLOGY CONFERENCE IN SAN ANTONIO, TEXAS

Histologic and morphometric evaluation of testes of feral tom kittens and cats

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Department of Animal and Rangeland Sciences, Oregon State University, Corvallis, OR

Background: Even with numerous successful Trap-Neuter-Release programs, feral cat populations continue to grow. Our laboratory is interested in determining if an underlying biological cause exists for the exuberant reproductive success in this once domestic subspecies. An earlier age for developing reproductive capacity (onset of spermatogenesis in males) may be one factor. For domestic toms, puberty is reported to occur around 8 months of age.1 Previous work by our laboratory has shown that normal morphologic sperm are present in vas deferens secretions of feral toms before 6 months of age.[2] Therefore, our hypothesis was that in feral toms the onset of spermatogenesis occurred before 6 months of age. The study objective was to histologically evaluate testes from weanling (2 months of age) through adulthood (24 months of age) to determine when the onset of spermatogenesis occurs in feral toms.
Methods: Feral toms were presented for castration at a local Humane Society during August-October 2014. Age was determined by records provided from feral cat colony managers and confirmed with dental eruption patterns. The age groups were: 2-2.5 months (weanling; n=6), 3-4 months (juvenile; n=6), 5-6 months (pubertal; n=6), and 12-24 months (adult; n=6). General anesthesia was induced and a routine open castration was performed. Both testicles from each cat were hemi-sectioned, formalin-fixed, paraffin-embedded, cut into sections (6 µm), and stained with hematoxylin and eosin. The slides were evaluated by a single observer (EB) blinded to age group using bright field microscopy (200X). Evidence of spermatogenesis was determined on the basis of presence of spermatozoa in the seminiferous tubule lumen. In addition, perpendicular diameters measured from 5 tubules for each testis were averaged and mean ± SD was determined for each age group. Tubular diameter was compared using a Student's t test where $p < 0.05$ was defined as significant. The presence of spermatozoa in the lumen was compared using a χ^2- test.
Results: Evidence of spermatogenesis in weanling, juvenile, pubertal, and adult toms was 0%, 17%, 67%, and 100%, respectively ($p < 0.05$ between successive age groups). The seminiferous tubular diameter was significantly larger in each successive age group (weanlings 88.10 ± 10.88 µm; juveniles 109.8 ± 8.89 µm; pubertal 142.2 ± 16.89 µm; adult 237.90 ± 52.45 µm).
Discussion: Juvenile feral toms in the current study had significantly wider tubular diameter than previously reported for domestic toms under 5 months of age (86 µm),[3] which supports our hypothesis that spermatogenesis is occurring at an earlier age in ferals. However, the time of year the previous measurements in domestics was not reported. If these measurements were made outside of the breeding season, this may explain why the tubular diameters were smaller. Future studies are planned to determine if folliculogenesis occurs earlier in queens as well.
Keywords: Castration, Feline, Puberty, Seminiferous Tubule, Spermatogenesis
References: [1] Tiptanavittana N., et al. *Reprod Fert Dev* 2014; 27(1):140.
[2] Bohrer E., et al. Comparison of penile spines and sperm morphology between juvenile and adult feral cats. *Society for Theriogenology Annual Conference Proceedings* 2014 (abstract).
[3] Sanchez B., et al. *J Reprod Fert Suppl* 1993; 47:343.

APPENDIX E. ABSTRACT PRESENTED AT THE 2016 RESEARCH ADVANCES IN FISHERIES, WILDLIFE, AND ECOLOGY SYMPOSIUM IN CORVALLIS, OREGON

Our laboratory is interested in determining if an underlying biological cause exists for the exuberant reproductive success observed in free-roaming unowned (FRU) cats. The study objective was to compare histological ovarian follicle classifications from FRU and owned cats at 2-6 months of age. Queens were presented to be spayed at a local Humane Society and grouped by age (<4 months (owned n=5, FRU n=10); 4-6 months (owned n=2, FRU n=7)). Age and life history data from cat colony managers were combined with dental eruption patterns to accurately estimate the age for FRU queens. A routine ovariohysterectomy was performed under general anesthesia. The total ovarian uterine weights from FRU queens were also recorded. Both ovaries were hemi-sectioned, formalin-fixed, paraffin-embedded, cut into sections (6 μm), and stained with hematoxylin and eosin. Slides were analyzed using bright field microscopy at 200X by a single observer (EB), blinded to the individual's age group and living status. Follicles were counted and classified as primary, secondary, or tertiary. Mean±SD percentages for each follicle classification were compared using a Student's t test. Total ovarian uterine weights were analyzed via linear regression. Significance was defined as $p<0.05$. FRU cats under 4 months old had more tertiary follicles compared to owned cats under 4 months of age (33% and 17%, respectively; $p<0.05$). As evidence of ovarian follicle endocrine function, total ovarian uterine weights were significantly higher in 4-6 month old FRU queens compared to under 4 months (1.18±0.31 g vs 0.93±0.28 g, respectively). Female FRU cats appear to be developing reproductive capacity at much earlier ages than what has been previously reported for owned domestic cats (~ 8 months). These observations may support why TNR efforts to reduce FRU cat populations are not successful. Selective pressures and a significantly shortened life span may be factors contributing to this finding.

APPENDIX F. ABSTRACT ACCEPTED FOR PRESENTATION AT THE 2016 SOCIETY FOR THERIOGENOLOGY CONFERENCE IN ASHEVILLE, N. CAROLINA AND THE 2016 NORTHWEST REPRODUCTIVE SCIENCE SYMPOSIUM IN CORVALLIS, OREGON

Early Onset of Reproductive Capacity in Free-Roaming Unowned Queens

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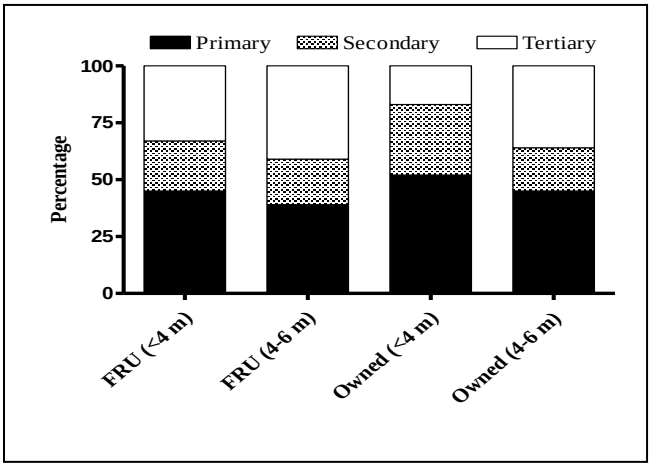
Background: Our laboratory is interested in determining if an underlying biological cause exists for the exuberant reproductive success observed in free-roaming unowned (FRU) cats. We have previously shown that FRU toms reach reproductive capacity by 4 months of age.[1] The hypothesis of the current study was that FRU queens had more tertiary follicles at a younger age compared to owned queens. The study objective was to compare histological ovarian follicle classifications from FRU and owned cats at 2-6 months of age.

Methods: Queens were presented for ovariohysterectomy at a local Humane Society in August and September 2015. Based upon previous observations in toms, queens were grouped by age (<4 months (owned n=5, FRU n=10) and 4-6 months (owned n=2, FRU n=7)). Age and life history data from cat colony managers were combined with dental eruption patterns to accurately estimate the age for FRU queens. A routine ovariohysterectomy was performed under general anesthesia. The total ovarian uterine weights from FRU queens were also recorded. Both ovaries were hemi-sectioned, formalin-fixed, paraffin-embedded, cut into sections (6 μm), and stained with hematoxylin and eosin. Slides were analyzed using bright field microscopy at 200X by a single observer (EB), blinded to the individual's age group and living status. Follicles were counted and classified as primary, secondary, or tertiary. Mean±SD percentages for each follicle classification were compared using a Student's t test. Total ovarian uterine weights were analyzed via linear regression. Significance was defined as $p<0.05$.

Results: FRU cats under 4 months old had more tertiary follicles compared to owned cats under 4 months of age (33% and 17%, respectively; $p<0.05$; see Figure). As evidence of ovarian follicle endocrine function, total ovarian uterine weights were significantly higher in 4-6 month old FRU queens compared to under 4 months (1.18±0.31 g vs 0.93±0.28 g, respectively).



Discussion: Both female and male FRU cats appear to be developing reproductive capacity at much earlier ages than what has been previously reported for owned domestic cats (~ 8 months).[2] These observations may support why TNR efforts to reduce FRU cat populations are not successful. Selective pressures and a significantly shortened life span may be factors contributing to this finding.

Keywords: Feral, Folliculogenesis, Ovary, Uterus

References: [1]Bohrer E, et al. Histologic and morphometric evaluation of testes of feral tom kittens and cats. *Society for Theriogenology Proceedings* 2015; abstract. [2]Jemmett JE, Evans JM. A survey of sexual behavior and reproduction of female cats. *J Small Anim Pract* 1977;18:31-37.

APPENDIX G. ABSTRACT ACCEPTED FOR PRESENTATION AT THE 8TH INTERNATIONAL SYMPOSIUM ON CANINE AND FELINE REPRODUCTION IN PARIS, FRANCE

Early Onset of Reproductive Capacity in Free-Roaming, Unowned Cats

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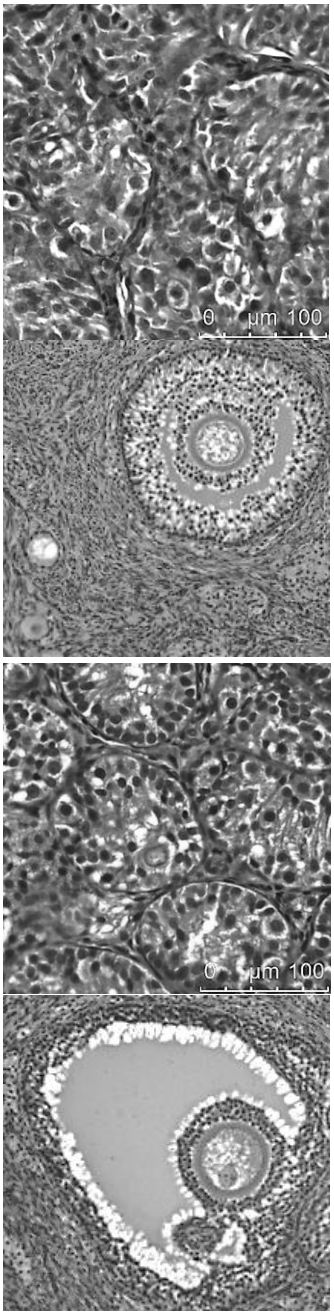
Free-roaming unowned (FRU) cat populations around the world continue to grow. Our laboratory is interested in determining if an underlying biological cause exists for the exuberant reproductive success observed in this animal. We have hypothesized that FRU cats are reaching reproductive capacity sooner than what has been previously reported in owned, domestic cats (~8 months) [1]. The objective of this study was to examine FRU kittens presented for surgical sterilization for reproductive markers (spermatogenesis in males and folliculogenesis in females). For males (under 4 months n=10; 4-6 months n=8), a routine castration was performed. For females (under 4 months (FRU n=10) and 4-6 months (FRU n=7)), a routine ovariohysterectomy was performed. In both genders, gonads from each cat were hemi-sectioned, formalin-fixed, paraffin-embedded, cut into sections (6 µm), and stained with hematoxylin and eosin. The slides were blindly evaluated at 200X by the same observer (KP for males; EB for females) using bright field microscopy. For males, a spermatogenesis scoring system was used [2]. For females, the largest follicle diameter was measured. Mean ± SD was determined for each parameter. Spermatogenesis score in under 4 month old and 4-6 months old FRU tomcats was 4.5±2.0 SD and 8.1±2.7 SD The largest follicle size in under 4 month old and 4-6 months old FRU queens was 581.6±53.6 SD µm and 469.4±113.9 SD µm. In the FRU cats studied, the onset of reproductive capacity appears to occur before 4 months of age in females but not until 4-6 months of age in males. We suspect that external stressors and a shortened life expectancy are contributing to this capacity for earlier reproduction. This finding is important for population control programs and veterinarians alike, as it suggests that sterilization efforts should be focuses on female cats under 4 months of age. Additional studies using FRU cats from other geographical areas are needed to determine if this observation is an isolated phenomenon or worldwide in distribution.

[1] Jemmett JE, et al. *JSAP* 1977;18:31-7.
[2] Yoshida A, et al. *Fertil Steril* 1997;68(3):514–518.



Representative gonadal cross-sections from a male and female FRU cats under 4 months (top) and 4-6 months old (bottom).