Sexual Dimorphism and Offspring Sex Ratio in Feral Cats

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Abstract

This pilot study investigated several reproductive parameters including anogenital distance and intrauterine position to determine its effects, if any, on fertility factors such as litter size and sex ratio in feral cats in the NC triad. While it is known that males possess larger anogenital distances across many mammalian species, some of the first averages on anogenital distance in feral cats were calculated in this study. The average anogenital distance on male cats in this study was found to be 2.22 cm and 1.29 for females (includes all females analyzed: pregnant and normal females). The data obtained in this study indicate that there is no statistically significant correlation between body mass and anogenital distance in neither males nor females. No conclusion could be made as to whether anogenital distance affected litter sex ratio due to sample size limitations of this study. With regards to anogenital distance and an effect on litter size, no statistically significant correlation was found between the two variables. Intrauterine position results were inconclusive, once again due to sample size limitations of this study. While more information is necessary before it can be determined whether the phenomenona of anogenital distance and intrauterine position are reliable predictors of pregnancy outcomes (including litter size and sex ratios), the results of this study demonstrates the lack of correlation between several variables and indicates the relationships between variables that require more data collection. It also established the sexual dimorphism of anogenital distance in feral cats.

Introduction

In 1973, the Trivers-Willard hypothesis proposed that a female animal's litter size and sex ratio may be affected by maternal condition [1]. Several studies have been conducted on mammalian species that expand on the Trivers-Willard hypothesis and the results have implied that a variety of factors have the potential to affect a given female's litter size and sex ratio, some related to maternal condition and others independent of it. One such factor is anogenital distance, a sexually dimorphic parameter used as a biomarker of prenatal androgen exposure. Anogenital distance for females is measured by taking the distance between the center of the vaginal opening to the center of the anus and for males, measuring the distance between the base of the penis and the center of the anus. Previous studies have demonstrated in mice that females with larger anogenital distances tend to have smaller, male-biased litters. Szenci et. al. tested this trend on wild mouse species and found that the correlation between the mother's anogenital distance and the litter sex ratio was strong, extending the findings of the previous mice studies that supported the hypothesis that anogenital distance is a factor affecting litter sex ratio [1].

Among large litter sizes, the intrauterine position of the mother is another factor to consider with regard to future litter size and sex ratio. The sexual differentiation process of females has been shown to be affected by adjacent male littermates, due to the testosterone the males produce. In mice, this phenomenon has been described by labeling the females as 2M, 1M, or 0M to quantify the number of adjacent male littermates, e.g., a 2M female had 2 adjacent male littermates [1]. Anogenital distance has been indicated as a reflection of intrauterine position. The 2M females tend to have longer AGD than 1M females, those being intermediate anogenital distances compared to 0M females which have the shortest AGD. These anogenital distance trends of 2M, 1M, and 0M females have been shown to hold at birth and in adulthood in many rodent species.

The mother's social status (a definite indication of maternal condition from an animal behavioral standpoint), has been observed in some studies to affect litter size and sex ratio with the general pattern being that high ranking females in the community tend to produce larger, male-biased litters. This trend suggests that the benefits of high rank include access to resources [1]. But, social ranking is not a factor that affects litter sex ratios in all mammalian populations.

Exposure to environmental pollutants including: xenoestrogens, pesticides, and heavy metals can have adverse effects on fetal development. The stage of fetal development at the time of exposure, length of exposure, and total dosage received of these chemicals determines the extent to which fetal development is affected (if at all). Xenoestrogens have been classified as endocrine disruptors and have the ability to mimic hormones or act as antagonist in order to disrupt hormonal pathways. When in-utero exposure to any of these xenoestrogens, pesticides, and heavy metals occurs, the generation of reactive oxygen species (ROS) follows. This oxidative stress contributes negatively to the health of the offspring and may cause embryonic death, spontaneous miscarriage, growth or development restrictions in utero, early delivery, and low birthweight [3]. A study conducted in 2011 found that in-utero exposure of human males (through parental occupational exposure) to BPA (Bisphenol-A), a common endocrine disruptor resulted in a shortened anogenital distance [4]. This finding was among the first evidence of BPA's adverse effects on development of male genitalia.

Endogenous hormones also play a key role in development and their abnormal secretion can result in improper sexual differentiation and disorders. Undescended testicles in males can result from decreased prenatal androgen secretion, stemming from either decreased pituitary gonadotropin stimulation or decreased production of placental gonadotropin [5].

Determining the physical reproductive parameters of a species is beneficial in gaining an understanding and being able to predict what physiological and behavioral effects will be present in a given animal population. For example, the testosterone release of prenatal male littermates discussed above is of special interest because data show that it can have prolonged effects on general health and social interactions in many species [1].

Readily accessible reproductive parameters include anogenital distance and intrauterine position, and these variables have been studied in mice, gerbils, rabbits and other mammals. Few data have been collected on feral cat populations to determine whether the previously discussed reproductive parameters have an influence on sex ratio and size of the litter. Data has been collected from spay/neuter clinics across the U.S. that record gender distribution, overall health conditions, and location of each cat that is spayed or neutered at the clinic.

The research I have conducted is an extension of previously conducted research on rodents to determine if an association exists between anogenital distance or weight and the sex size/ratio of a litter in feral cat populations. Cats have been used in biological studies for many years as model organisms, especially in neuroscience. Cats are reflex ovulators, meaning that the ova are released from the ovaries reflexive to mating and does not occur in the absence of mating. Cats usually have a heat cycle that occurs seasonally, with most conceptions in the spring season [7]. Depending on location breeding season typically begins in January and can last until September [6].

The prenatal development of a kitten is complex and many important milestones occur during gestation. Some key points in development particularly relevant to this study are: days 18-19-forelimbs and hindlimbs begin to develop, day 21-the genital system is present in its primitive form, and days 32-38-the external genitalia are completely formed. Day 38 is considered the end of the second trimester and the total gestation period of these animals lasts somewhere between 60-63 days [8]. With regards to the early stages of external genitalia development, there are data to suggest that segments of external genitalia are generated in the lower ventral surface of the abdominal wall; the genital tubercle, cloacal folds, and genital swellings are visible as faint elevations between days 21-32. The cloacal folds encompass the urogenital folds and the anal folds, subdivisions of the primitive cloaca. The genital tubercle develops into the penis and urogenital folds lift to form the scrotum in males; the clitoris, labia minora, and the labia majora are formed from the genital tubercle and urogenital folds [9].

The anogenital distance has been used as a method for determining the sex of cats since male cats have significantly longer anogenital distances than females. Other than sex determination, it is not known if anogenital distance provides any other information in cats regarding reproductive parameters. While maternal condition (nutrition, absence of disease, etc.) might affect a female's litter, it has not been documented whether or not the anogenital distance and/or body weight of a mother cat have any influence on the litter size or sex ratio.

Methods

Feral cats for this study were obtained by working "spay days" with the Feral Cat Assistance Program under the direction of Dr. Karyn Waterman. This local organization serves the piedmont NC area by spaying and neutering feral cats trapped by volunteers. Following the spaying or neutering procedure, the anogenital distance of the cat was taken with microcalipers to the nearest hundredth of a centimeter and recorded. For pregnant cats, the uterus was examined, the number of fetuses was counted, and if the sex ratio of the litter could be determined, it was recorded. The body mass of all cats recorded in this study was recorded with a digital scale.

This pilot study sampled 84 feral cats, 20 males and 64 females. 18 of the females were pregnant. The duration of the study was six months (September 2014-March 2015) and the study sample size was limited to the number of cats trapped by the volunteers each month and the technical aspect of collecting the data without disturbing the rest of the process of the clinic.

Results

From all of the feral cats evaluated in the study, the following mean ± SEM data were calculated:

Average Male AGD (n=20)	2.22 cm (± 0.10)
Average Female AGD (n=64)	1.29 cm (± 0.08)
Average Pregnant Female AGD (n=18)	1.51 cm (± 0.07)
Average Male Fetal AGD (n=13)	0.50 cm (± 0.02)
Average Female Fetal AGD (n=5)	0.45 cm (± 0.05)

It should be noted that the pregnant female average anogenital distance (AGD) is 0.22 cm longer than normal (non-pregnant) females. There is a significant difference between male and female anogenital distance, 0.93 cm between males and females of normal reproductive status and a 0.71 cm difference between males and pregnant females. The pattern of larger anogenital distance in males than females also held in the fetal kittens with a 0.05 cm difference between male fetal kittens and female fetal kittens.

Of the 64 females sampled, body mass and anogenital distance were measured in an attempt to discern any relationship between these two variables. The correlation between body mass and anogenital distance was not statistically significant (Fig.1). A previous study conducted on two different species of mice produced the same result: anogenital distance and body mass were not correlated in adult mice. The study did find that anogenital distance and body mass were correlated in pre-pubertal mice, but this relationship did not continue post-puberty (Szenci, 2013). The same measurements were taken on the male feral cats brought to the clinic and again, no correlation between body mass and anogenital distance was demonstrated (Fig. 2). These results support knowledge that anogenital distance is determined by fetal androgenization and developmental factors in feral cats, like its manifestation in other mammalian species.

Of the pregnant cats analyzed, only 8 had pregnancies that had progressed far enough to determine the sex ratio. Because of this small sample size, no statistically significant difference could be drawn with regards to anogenital distance of female feral cats and the sex ratio of their litter. The data collected display no statistically significant correlation between these two variables, but with a much larger sample size it is possible that a statistically significant correlation could be discovered between longer maternal anogenital distances and male biased litters (Fig. 3). One trend noted here is that small litters (3 kittens or less) tended to be male biased. More data are necessary to confirm this trend.

The number of kittens in a litter was not correlated with the maternal anogenital distance at a statistically significant level. Some of the mother cats with smaller anogenital distances were gestating large litters (5 kittens or greater) and some of the mothers with larger anogenital distances had relatively small litters gestating. This supports the findings of Szenci and others who found that anogenital distance and litter size are unrelated (Fig. 4).

Due to the very small sample size of litters that allowed for sex determination there was not much information gathered that supported any real effect of intrauterine position and fetal anogenital distance. Many more cats should be surveyed, more uteruses collected and measured, and a more accurate means to estimate the progress of the pregnancy are needed in order to truly understand intrauterine position's role in the determination of anogenital distance.

Discussion

Alley Cat Allies, an organization that operates out of Virginia has established a monthly spay/day clinic that serves cats from Delaware, Maryland, Virginia, West Virginia, and DC and collects data on each cat that receives their services. This organization's findings are consistent with peer-reviewed studies of other feral cat spay/neuter clinics across the country. They have found that slightly more female cats are brought to the clinic than males and that of those female cats, close to one in every five was pregnant [6].

Among the data collected in this study, were some of the first average anogenital distances on feral cats in the NC Triad area. It was found that the average anogenital distance in the male feral cats sampled was 2.22 cm, and in the female feral cats sampled the average was 1.29 cm. The average of only pregnant females was 1.51 cm. These data demonstrate the sexual dimorphism of anogenital distance in feral cats. Male cats have significantly longer anogenital distances than females regardless of the reproductive status of the female. The study allowed for the gathering of preliminary statistics on the aforementioned reproductive parameters (anogenital distance, intrauterine position, sex ratio). More information will be needed before making the case that these reproductive traits affect pregnancy outcomes as is noted in mice and many other rodents.

As this study was newly designed there were some aspects that need to be added and others that need to be improved upon if it is to be continued. These include: collecting the mass of the fetal kittens, determining the stage of development (genitalia formed/not formed) and dividing the collected data into categories based on the stages. This could assist in identifying relationships that were not found in this study due to the fact that all data was analyzed together since samples obtained were solely based on the number of cats brought to the clinic in the given six months that the study took place. The timeline of the study needs to be expanded significantly in order for more statistically significant and apparent results since sample collection is random and completely dependent upon volunteers trapping cats and bringing them into the clinic.

The data collected during this study, once expanded upon, could reveal patterns in feral cat reproduction, extend what is known about mammalian reproductive parameters, including anogenital distance and intrauterine position.

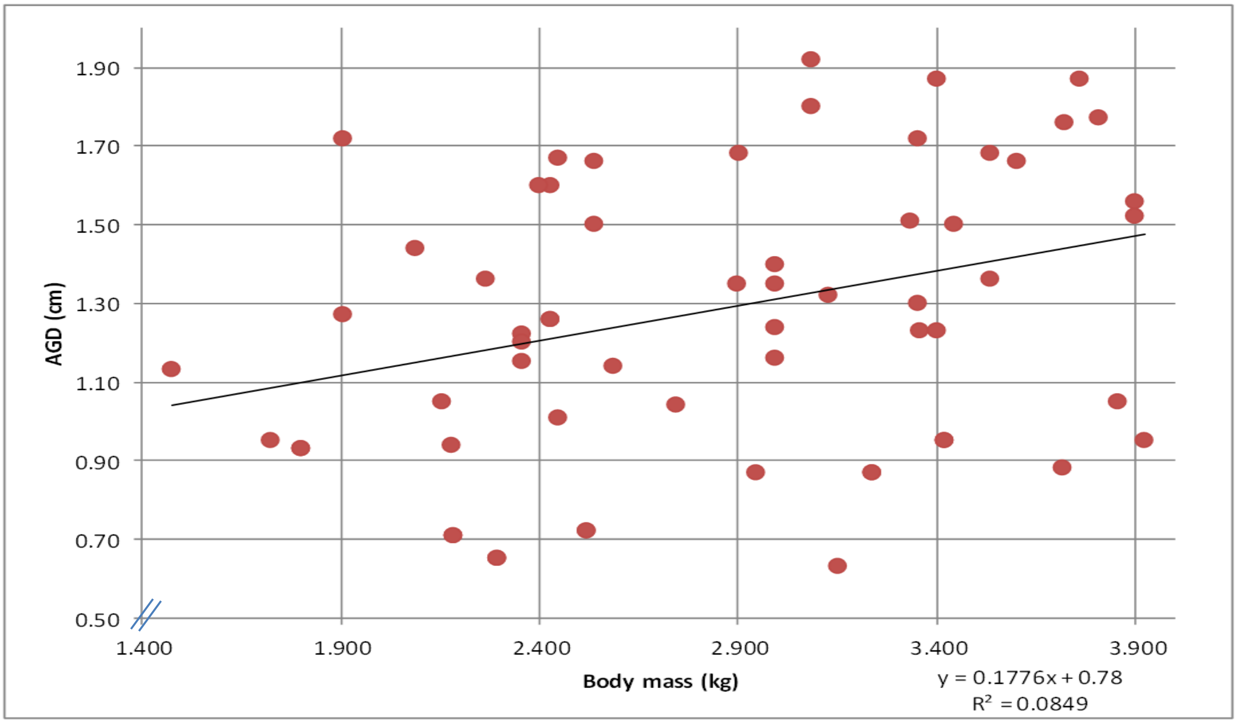
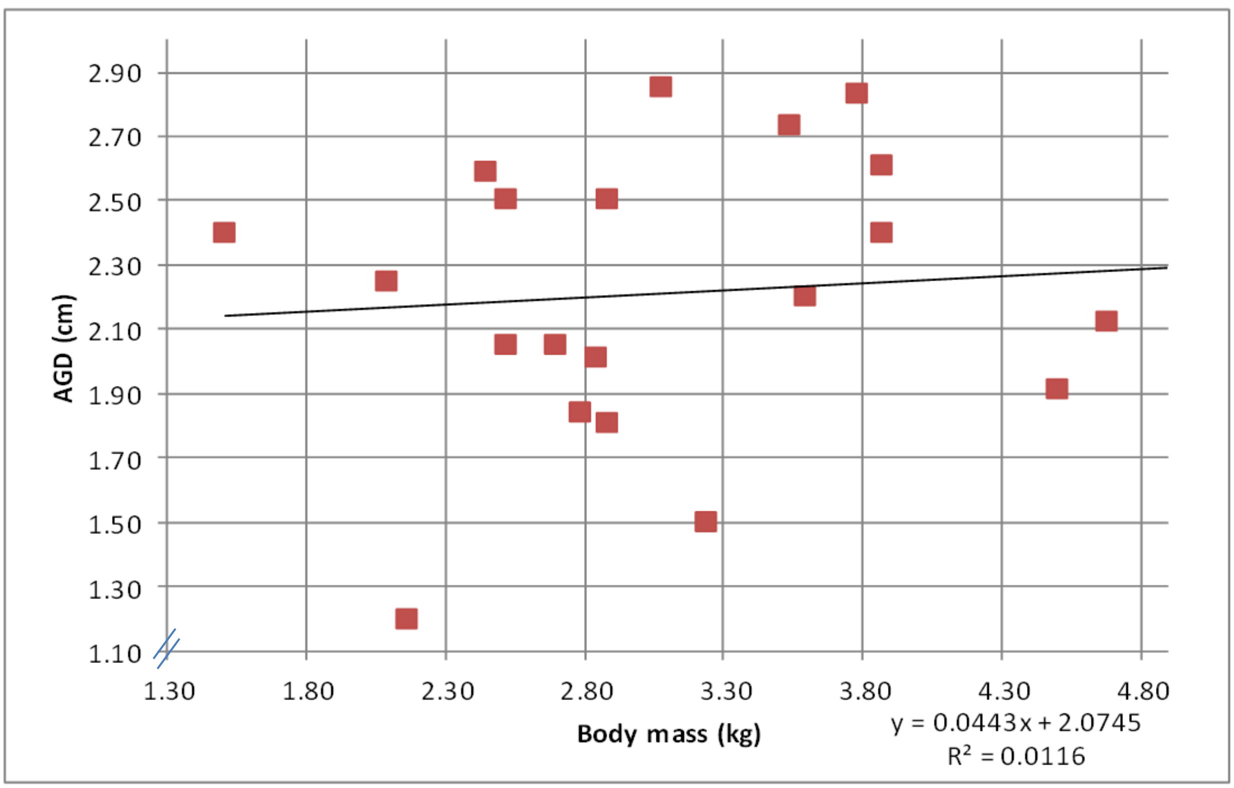


Figure 1 (above): Displays body weight in kilograms and anogenital distance in centimeters of each female cat sampled in this study, p value = 0.265. **Figure 2 (below):** Displays body weight in kilograms and anogenital distance in centimeters of each male cat sampled in this study, p value = 0.477.



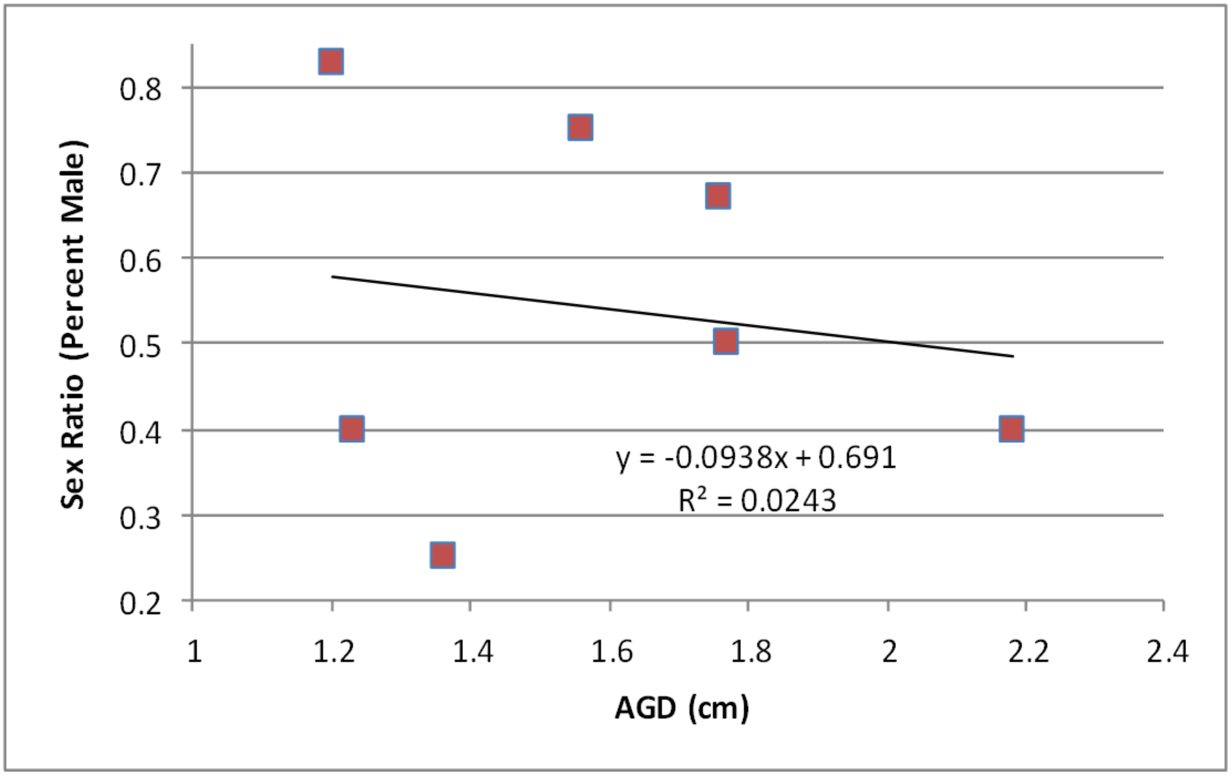
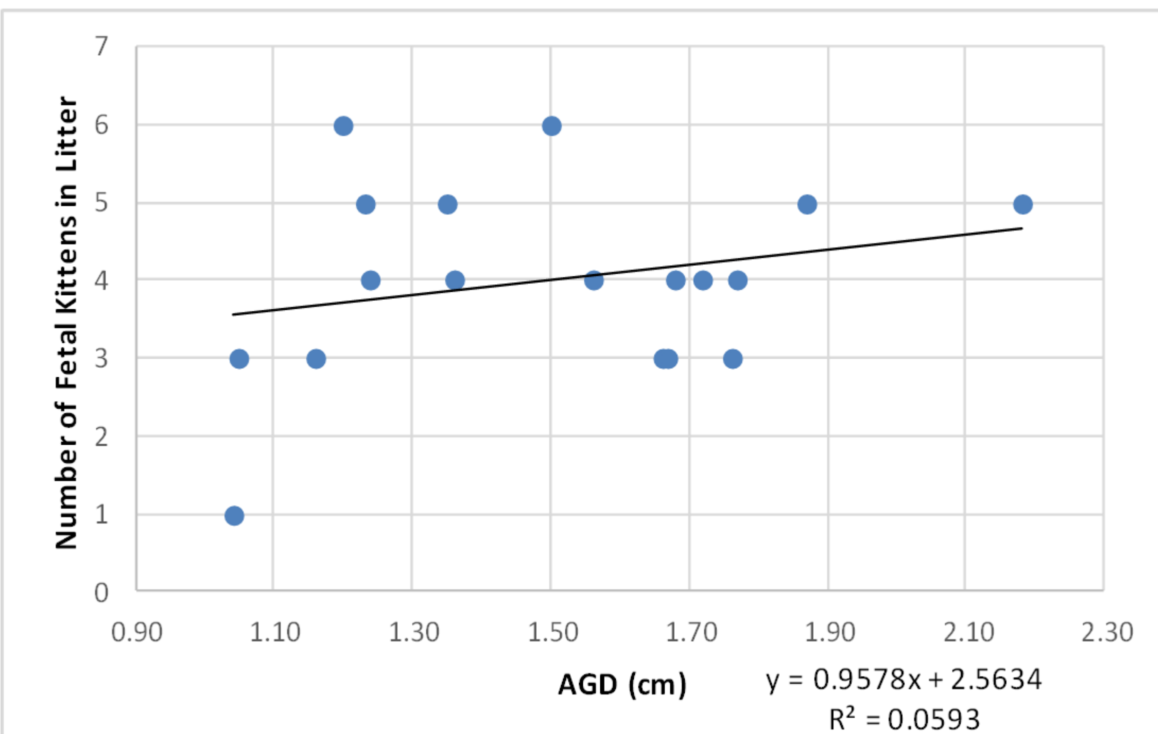


Figure 3 (above): Displays the anogenital distance (cm) and the sex ratio of the litters that were developed enough to be determined, p value = 0.481**Figure 4 (below):** Displays the anogenital distance of each pregnant female cat in the study and number of kittens in each litter, p-value = .421



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