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**PARTNER PREFERENCE AND SEXUAL PERFORMANCE
IN MALE GOATS, CAPRA HIRCUS**

by

ANDREA M. HAULENBEEK

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ABSTRACT OF THE DISSERTATION

Partner Preference and Sexual Performance in Male Goats, *Capra hircus*

By ANDREA M. HAULENBEEK

Dissertation Director:

Larry S. Katz

Sexual performance is a combination of the physical ability to mate and sexual motivation. An indirect measure of sexual motivation is partner preference, because sexual motivation can be stimulated by the presence of suitable mates. We hypothesized that the tail wagging behavior of female goats in estrus contributes to their incentive value, thus affecting partner preference and sexual performance in males. A previous study in our laboratory showed that flutamide, an androgen receptor antagonist, enhanced tail wagging in estrous females, as it did in the current studies. Partner preference testing was used in which sexually experienced males could choose between two females. Females were non-estrous (NE), estrous (E) or flutamide-treated estrous (E_F). Males showed no preference for an E versus a NE female and preferred an E_F female more than an E or a NE female. Males also visited the E_F female the most. Tail wagging initiated male approach behaviors and maintained the attention of the male, therefore, tail wagging behavior is both attractive and proceptive. Furthermore, we hypothesized that exposure to females expressing high rates of tail wagging would arouse males, increasing sexual performance. Sexually experienced males observed different stimuli before a sexual

performance test: an empty pen, or groups of three females that were all E, NE, E_F, or non-estrous and treated with flutamide (NE_F). Viewing E_F females increased the number of ejaculations attained by males and decreased the latencies to first and second ejaculation, as well as the inter-ejaculatory interval. Viewing estrous females (E and E_F), as compared to non-estrous females (NE and NE_F), decreased the latency to first mount. Another study revealed that partner preference is dependent on the male's hormonal state and not his sexual experience. Males were tested for partner preference with a choice of groups of E or NE females. Sexually naive castrates showed no preference. Sexually naive or experienced intact males and sexually naive TP-treated castrates preferred E females. Partner preference, however, is not a reliable indicator of sexual performance in male goats, as there were no significant correlations between sexual performance and partner preference for sexually naive or experienced males.

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INTRODUCTION

Domestic farm animals, including goats, are important to the world economy. Gaining a better understanding of animal reproduction is essential for continued success, especially with regards to meeting an increasing world demand for food. The success of animal industries relies on the reproduction of animals, which is dependent upon the willingness and ability of animals to engage in reproductive behavior. Reproduction can be optimized through a better understanding of the constituents of sexual behavior, such as sexual performance and motivation. Understanding what motivates a male goat to engage in sexual activity could increase the efficiency and productivity of breeding programs, which can also be applied to similar species, such as cattle.

Sexual performance tests given to young males may be inaccurate in evaluating future sexual performance because physical and psychological factors affecting sexual behavior develop at different rates. Sexual motivation may be well-developed early, but inadequate body size and lack of strength, endurance and coordination may impair performance. Further, juvenile sexually naive goats display separation anxiety when removed from pen mates and require up to six sexual interactions with receptive females before attaining a consistent serving capacity (Imwalle and Katz, 2004a). Sexual motivation in young males may be a better predictor of adult performance, particularly if brain systems underlying motivation develop earlier than physical systems mature. Partner preference is one method to easily and indirectly assess sexual motivation. Identifying replacement breeders when males are still young would increase the efficiency and productivity of breeding programs. Money would be saved by identifying

replacement males earlier, cutting costs to maintain a larger group of males until old enough to be tested using sexual performance tests. Furthermore, the ability to identify high performing males would allow conceptions earlier in the breeding season. This allows larger offspring to be sent to market resulting in larger profits.

Goats are important to agriculture for their meat and their milk. As of January 1, 2007, there were 2.93 million meat and dairy goats in the United States, up 3 percent from 2006 (NASS, 2007). Goats and cattle share similar sexual behavior. The goat can serve as a cost-effective model for cattle because they reach sexual maturity faster and the cost of maintaining a research herd is less expensive. The United States is the world's largest beef producer, and milk has a farm value of production second only to beef among livestock industries (USDA, 2008). Not only is the U.S. goat population on the rise, but this trend is also seen throughout the world, with goat populations increasing 26.4% between 1993 and 2003 (Boyazoglu et al., 2005). World goat populations have also seen the largest increase among ruminants with a 56.3% comparative change from 1983 to 2003, while sheep populations are down 8.6% and cattle populations are up only 9.6% (Boyazoglu et al., 2005).

Dairy goat farming is important to the national economies of many developed countries, including France, Greece, Italy, and Spain (Boyazoglu et al., 2005). There has been an increased market demand for goat cheese and yogurt, as it is now viewed as gourmet in some locations (Haenlein, 2007). Goat milk is also receiving elevated status due to its exceptional nutritional value (Haenlein, 2004). Not only are goats important for their meat and their milk, but interest in fibers, skins, and brush control for fire prevention or to create better pasture for sheep and cattle to graze, has generated renewed

interest in the species (Boyazoglu et al., 2005; Haenlein, 2007). In general, goat products have a healthy and ecological image that is often associated with agro-tourism in many mountainous regions (Dubeuf et al., 2004). In addition, the goat is a culturally important animal that is used for religious and cultural events, as opposed to one of purely economic interest (Alexandre et al., 2002).

Goat farming is also important to developing nations. Developing nations, especially the poorest of these countries, have seen a continuous and rapid increase in goat populations and products, which “indicates that this animal might provide the tool required to meet some of the needs accompanying the continuous increase of human populations” (Boyazoglu et al., 2005). The resilience that goats show under harsh conditions, namely adaptability to excessive hot and cold temperatures, high altitude levels, underfeeding, ability to walk long distances, drought survival, and resistance to mosquito-born tropical diseases, substantiate this idea (Morand-Fehr, 1988; Haenlein, 2001; Iniguez, 2004; Boyazoglu et al., 2005).

In addition to their importance to agriculture, the goat can serve as an additional model for the study of behavioral endocrinology. The majority of such research, in particular the study of sexual motivation, has been conducted with rodents. The goat may serve as a better model than laboratory rodents for understanding the behavioral endocrinology of humans for various reasons: 1) goats lack the level of inbreeding seen in laboratory rodents; 2) goats possess a more complex behavioral repertoire than rodents; 3) goats display both homotypical and heterotypical behaviors, similar to humans. For example, male and female goats will mount both sexes.

The overall aim of this project was to study partner preference, an indirect measure of sexual motivation, and sexual performance in male goats. In order to do so, we had to identify a method that can measure partner preference in both sexually experienced and sexually naive males, as well as identify female characteristics that are important for partner preference and sexual motivation in male goats. Preliminary experiments (see Appendices A-C) determined an appropriate test to measure partner preference in male goats, and suggested the tail wagging behavior of female goats in estrus may play an important role in male partner preference and sexual behavior. The objectives of this project were (1) to determine if males prefer the tail wagging behavior of estrous females; (2) to determine if the tail wagging behavior of estrous females can stimulate sexual performance in males; (3) to determine if male preference for the tail wagging behavior of estrous females is dependent on hormonal state or sexual experience of the male; and (4) to determine if strength of preference for estrous females can predict sexual performance in sexually experienced and sexually naive males.

CHAPTER 1

The Study of Sexual Behavior

Niko Tinbergen (1963), one of the founders of modern ethology, stated that in order to study behavior, one must first properly observe and describe the behavior. Then, four kinds of questions can be asked about the behavior: immediate causation, development, evolutionary history and function. The study of behavior is aided by its division into two phases: appetitive and consummatory (Craig, 1917). Appetitive behaviors are the more variable, searching phase of a behavioral sequence and consummatory behaviors are the more stereotyped behaviors that lead to the extinction of a behavioral sequence (Ball and Balthazart, 2008).

Frank Beach is considered the founder of behavioral endocrinology. Beach (1956) brought the concept of appetitive and consummatory behaviors to the study of sexual behavior, observing that sexual motivation and performance are parts of two different mechanisms. For sexual behavior, the appetitive phase involves seeking sexual encounters and includes courtship behaviors, which are behaviors exhibited by an individual in an attempt to gain access to an individual of the opposite sex for the purpose of mating. This phase allows for information to be exchanged between individuals, such as readiness to mate and genetic information (Nelson, 2005). Sexual motivation is a behavioral component of the appetitive phase. The consummatory phase encompasses copulation, including sexual performance. The division of the two phases can be useful for heuristic purposes, as it provides a distinction between seeking sex (sexual motivation) and successfully mating (sexual performance) (Nelson, 2005). The

limitations of this division are noted by Beach (1976) in that appetitive and consummatory behaviors often alternate during actual mating, and that the same behavior, such as assuming a coital posture, can be appetitive or consummatory depending on the circumstances of the behavior. Pfaus (1996) proposed by that appetitive and consummatory phases can be thought of as overlapping Venn diagrams, which illustrate that the division between phases is not necessarily fixed and that some behaviors can be placed in both phases. Nonetheless, the distinction between appetitive and consummatory behaviors are the foundation of the study of sexual behavior.

Sexual Motivation

Beach (1956) proposed one of the first modern models of male sexual behavior; a two-factor theory which consisted of the appetitive sexual arousal mechanism (SAM) and the consummatory intromission and ejaculation mechanism (IEM). Beach theorized that copulatory behavior was initiated by the SAM and that it was subject to learning. In the following years, the SAM became equivalent to sexual motivation (Sachs, 1978; Agmo, 1999). Furthermore, sexual motivation is stimulated by the presence of a suitable mate (Beach, 1956). Approach behaviors by the male are activated by appropriate incentives by the female, therefore sexual motivation is an incentive motivation.

Many studies have been conducted to examine aspects of sexual motivation. These studies demonstrate that males will perform tasks or overcome obstacles to gain access to a receptive female. One of the earliest studies of sexual motivation showed that male rats would cross an electrified grid to gain access to a receptive female (Moss, 1924). Also, male rats will overcome other aversive stimuli or obstructions (Warner,

1927; Jenkins, 1928; Stone et al., 1935; Anderson, 1938; Meyerson and Lindstrom, 1973), dig through sand (Anderson, 1938), or turn a wheel (Denniston, 1954) to gain access to a receptive female. Rats will also engage in nose-poking and other attempts to “get to” females located behind a wire-mesh screen (Pfaus, et al., 1990; Damsma et al., 1992; Pfaus, et al., 1995).

Sexual motivation has been studied with operant paradigms, such as bar pressing in male rats (Schwartz, 1956; Beck, 1971; Jowaisas et al., 1971; Beck, 1978; Everitt et al., 1987; Everitt and Stacey, 1987). Male rhesus monkeys also will bar press to gain access to a receptive female (Michael and Keverne, 1968). Similarly, male pigeons will key peck (Gilbertson, 1975). Straight-arm runways also have been used to study sexual motivation in various species (Seward and Seward, 1940; Sheffield et al., 1951; Denniston, 1954; Kagan, 1955; Beach and Jordan, 1956; Whalen, 1961; Ware, 1968). The time it takes the subject to reach a goal box containing a stimulus is a measure of its motivation toward that particular stimulus. Sexually naive and experienced male rats run significantly faster for an estrous female than a non-estrous female (Lopez et al., 1999). Mazes (e.g. Y-maze or T-maze) have also been used to assess sexual motivation in male rats (Kagan, 1955; Whalen, 1961; Drewett, 1973; Hetta and Meyerson, 1978).

The aforementioned behaviors can be reduced following castration, administration of androgen receptor antagonists, or lesions of certain steroid-concentrating brain regions, such as the basolateral amygdala (Nissen, 1929; Everitt et al., 1987). This indicates that gonadal steroid actions in the brain are necessary for the development and/or maintenance of these behaviors (Pfaus et al., 2003). However, sexually experienced males are relatively resistant to many treatments that disrupt sexual

behavior in inexperienced males, including castration, penile anesthesia, novelty of a testing chamber, olfactory deprivation, and age (Lodder, 1975; Thor and Flannelly, 1977; Lisk and Heiman, 1980; Gray et al., 1981; Pfaus and Wilkins, 1995; Pfaus et al., 2001).

Preference paradigms are also employed as an indirect measure of sexual motivation. A partner preference test simultaneously exposes the subject to various stimuli in a maze or a test pen. Time spent in proximity to a stimulus or the total number of times the subject chooses a stimulus are measures of preference for that stimulus. Sexually naive and experienced male rats spend more time near sexually receptive versus non-receptive females or males (Meyerson and Lindstrom, 1973; Hetta and Meyerson, 1978; Merx, 1983; Edwards and Einhorn, 1986; Agmo, 2003). Castration abolishes these preferences and testosterone replacement restores them (Agmo, 2003).

The incentive value of female cues varies by species. For instance, olfactory cues are important determinants of the incentive value of female rodents. Sexually naive and experienced male rats display preferences for the odors of sexually receptive versus non-receptive females (Carr et al., 1970; Stern, 1970; Bakker et al., 1996). Sexually naive male ferrets show no preference between a male and a sexually receptive female or between their odors, but sexual experience elicits a preference for a sexually receptive female or her odors (Kelliher and Baum, 2002). On the other hand, sexually experienced bulls do not show a preference for an estrous female when the main difference between females is olfactory cues. Bulls show no preference between a sexually receptive and non-receptive female, but prefer a pair of sexually receptive females engaging in female-female mounting versus either a pair of sexually receptive females unable to mount or a pair of non-receptive females (Geary et al., 1991; Geary and Reeves, 1992). Therefore, it

appears that visual cues play a greater role than olfactory cues in the incentive value of female cattle.

These species differences in the incentive value of female cues can be attributed to the environment in which each species lives. Ferrets and rats are both nocturnal animals that rely on olfaction in dark, enclosed spaces rather than vision to find a mate. Cattle and goats live in herds in which females form groups in the center of the home range and adult males live on the periphery (Geist, 1964; Crook, 1969; Kilgour et al., 1977). When in estrus, female goats and cattle form sexually active groups that engage in female-female mounting, as well as other behaviors (Shank, 1972; Kilgour et al., 1977). For goats and cattle, visual stimuli may be more important in locating estrous females, as olfactory cues may not travel great distances across a home range. Female goats in estrus tail wag. Previous work in our lab suggests that tail wagging is a behavior that may serve to visually attract males from a distance (Imwalle and Katz, 2004b). This behavior may play an important role in the incentive value of the female goat and therefore sexual motivation in males.

The role of visual stimuli in the incentive value of females has been studied in other species in which females have developed pronounced secondary sexual characteristics that attract males, such as the sexual swellings of many Old World monkeys (Setchell and Kappeler, 2003). Many of these studies focus on the evolutionary process of sexual selection, described by Darwin (1871) as selection that “depends on the advantage which certain individuals have over others of the same sex and species solely in respect of reproduction.” This will be discussed further in the subsequent section “Female Sexual Behavior – Sexual Selection in Females.”

Sexual Performance

As previously noted, the consummatory phase of sexual behavior encompasses copulation. Consummatory behaviors are more stereotyped and species-specific than appetitive behaviors. This section on sexual performance will focus on studies in domesticated ruminants since the experiments in this thesis are concerned with the sexual performance of goats. Much research has been conducted on the sexual performance of cattle due to the negative economic impact of low performing males on this industry (Katz, 2007).

Anderson (1945) distinguished between two components of bull sexual behavior: libido and the ability to copulate. Hultnas (1959) defined libido as the willingness and eagerness to mate, much like Beach's (1956) definition of sexual motivation. Hultnas also noted that mating ability is separate from libido and is affected by motor patterns and anatomy. However, male sexual performance is typically measured as a combination of motor skills and sexual motivation (Price, 1987).

Petherick (2005) notes that a variety of tests to measure the sexual performance of male cattle have been developed during the last 30–40 years. These tests determine the sexual responsiveness of bulls to females, such as the latency for bulls to copulate (Amann and Almquist, 1976; Chenoweth, 1981; Landaeta-Hernandez et al., 2001); counts and durations of 'interest', such as sniffing at the vulva and time spent with females (Chenoweth et al., 1979; Coulter and Kozub, 1989; Bertram et al., 2002). Although these tests were designed to measure sexual performance, they are actually

measures of sexual motivation. There appears to be a disconnect between experimental and applied animal behavior research.

Research to identify endocrine predictors of sexual performance, such as correlating levels of testosterone, prostaglandin $F_{2\alpha}$ and luteinizing hormone to sexual performance, have been unsuccessful (Foote et al., 1976; Chenoweth et al., 1979; Price et al., 1986; Alexander et al., 1993; Alexander et al., 1999; Roselli et al., 2002). Other measures of sexual performance are the numbers of mounts and/or services during a set period of time (Blockey, 1981a; Coulter and Kozub, 1989; Landaeta-Hernandez et al., 2001; Bertram et al., 2002) and scores assigned according to various combinations of these measures (Chenoweth et al., 1979; Blockey, 1981b; Chenoweth, 1981; Landaeta-Hernandez et al., 2001). These sexual performance tests are generally termed serving capacity tests, and they assess sexual performance in terms of mating ability. The sexual performance of goats is assessed using serving capacity tests. Generally, a male is given access to one or more estrous females. The total number of mounts and ejaculations are recorded; the higher the number of ejaculations, the higher the male's sexual performance.

Observing sexual behavior before a sexual performance test can stimulate the sexual performance of male goats, cattle, pigs, and horses. Male horses with low or abnormal sexual behavior were more motivated to mount and mate with females when given the opportunity to observe other males and females mating prior to testing (Pickett et al., 1977). Male pigs exhibit shorter times to first mount after watching other males mount a dummy female (Hemsworth and Galloway, 1979). Male cattle exhibit enhancement of sexual performance when allowed to watch conspecifics mating (Mader

and Price, 1984). The male cattle were also stimulated when being watched by another male while copulating, but to a lesser degree than when they themselves had observed copulations. Mader and Price (1984) stated that sexual stimulation by social facilitation could give males a competitive advantage in multi-male breeding groups.

Similar results have been reported in male goats. Sexual performance of male goats is enhanced after viewing conspecifics mating (Price et al., 1984). Male goats were also stimulated, but to a lesser degree, when watched by another male in close proximity while mating. The sexual performance of male goats is also enhanced when males view females mounting one another before a sexual performance test (Shearer and Katz, 2006). Again, these findings point to the importance of visual stimuli in the sexual behavior of goats. Perhaps female tail wagging can stimulate sexual performance in male goats.

In contrast, male sheep do not exhibit enhancement of sexual performance when allowed to watch conspecifics mating (Price et al., 1991). Price et al. (1991) suggested two factors that may explain why male sheep are not sexually aroused by visual stimuli as are male goats and cattle. First, female sheep in estrus do not engage in female-female mounting, as do female goats and cattle. Further, female sheep display ram-seeking behavior when in estrus. Second, estrous sheep remain receptive after repeated mating, whereas, female goats and cattle experience diminished sexual receptivity after repeated mating. This second factor allows male sheep to take longer to locate and identify estrous females while still having the opportunity to successfully mate (Price et al. 1991).

Maina and Katz (1997) investigated the hypothesis that exposure to a recently mated male would increase the sexual performance of male sheep, as olfactory cues may play a more important role in the sexual behavior of sheep. They found that when males

interacted with a pen-mate that had recently mated with a female, there was increased olfactory investigation of that male. Furthermore, the investigating pen-mates showed increased sexual performance during a sexual performance test with females.

Sexual Behavior in Goats

Goats are a promiscuous species. In the wild, goats live in sexually segregated herds. Females and young live in the center of the home range, while adult males live on the periphery (Crook, 1969; Geist, 1964). When in estrus, female goats form sexually active groups that display certain behaviors, such as tail wagging and female-female mounting (Shank, 1972). Most goats are seasonal breeders, with the breeding season initiated by the decrease in day length that occurs during the fall-winter season in the northern hemisphere (Bissonnette, 1941). The breeding season usually occurs from late August through January, with peak estrous activity occurring from September through November when the day lengths are decreasing rapidly (Amoah et al., 1996). The experiments reported in this thesis were all conducted during the breeding season. The estrous cycle of the female goat is approximately 19 to 24 days in length (Shelton, 1978). The length of estrus varies by breed. French Alpine goats, which are the subjects of this thesis, display estrus for approximately 12 to 36 hours (Camp et al., 1983).

Table 1 displays the behaviors commonly performed by goats (Geist, 1964; Shank, 1972; Kaplan and Katz, 1994). These behaviors may be performed by both males and females in either a social or sexual context; tail wagging, however, is only performed by females in estrus. These behaviors will be discussed further in the following sections on female and male goat sexual behavior.

Female Sexual Behavior

Beach (1976) defined three categories of female sexual behavior: attractivity, proceptivity, and receptivity. This arbitrary categorization allows for a more refined measurement of behavior and also aids in differentiating between the consummatory and appetitive phases of female sexual behavior.

Attractivity describes the female's ability to stimulate interest from a male. It is typically measured by observing the behavior of the male, such as approaching and investigating the female, and is dependent upon the male processing primarily visual and olfactory cues provided by the female (Beach, 1976). For female goats, attractivity can be measured by the number of leg kicks, ano-genital sniffs, or mount attempts received from a male (Kaplan and Katz, 1994; Billings and Katz, 1997). Attractivity is highly influenced by ovarian hormone levels and is most intense when estradiol is secreted in high concentrations just prior to ovulation. This temporal relationship likely functions to increase the probability of a female attracting a mate and mating when she is most fertile (Beach, 1976). However, Beach notes that attractivity relies on more than just the hormonal state of the female. For example, a group of estrous females may display the same level of receptivity yet males may show preference for specific females. Preliminary studies (see Appendix C) revealed that estrous females displayed different levels of tail wagging (not quantified) and that males appeared to prefer the females that tail wagged more. Therefore, tail wagging may enhance a female's attractiveness, thereby affecting sexual motivation and partner preference in males.

Proceptivity is the sum of sexual behaviors exhibited by the female towards the male in order to initiate or maintain sexual interaction with the male and is typically measured by observing the behavior of the female, such as the ram-seeking behavior of sheep (Beach, 1976). Beach (1976) further points out the limitations of arbitrary categorizations because female proceptivity resembles her attractivity as it consists of appetitive behaviors from the female in response to stimuli from the male. If the female does not display proceptive behaviors it is likely that her attractivity would be decreased.

For female goats, proceptivity can be measured by the number of leg kicks, mounts, mount attempts, or tail wags by the female directed towards the male (Kaplan and Katz, 1994; Billings and Katz, 1997; Imwalle and Katz, 2004b). Females likely mount males to encourage the males to mount the females in return (Beach, 1976). Female-female mounting is another proceptive behavior displayed by female goats, which has been shown to stimulate the sexual performance of males (Shearer and Katz, 2006). Perhaps tail wagging may also stimulate the sexual performance of males.

Female goats display proceptive behaviors more fully when there is no male present or when the male's ability to interact with the female is reduced (Billings and Katz, 1999). Female goats in estrus form sexually active groups that engage in female-female mounting, as well as other behaviors (Shank, 1972). It is thought that tail wagging and female-female mounting by female goats may serve as visual cues to attract males from a distance (Imwalle and Katz, 2004b; Shearer and Katz, 2006).

Receptivity is defined as the female's readiness to copulate and is most obviously indicated by the female's immobility and receptive posture (Beach, 1976). A sexually receptive female will assume a posture to facilitate successful intra-vaginal intromission

and ejaculation. For a female goat, receptivity is measured by the number of mounts or mount attempts she stands to receive from a male (Kaplan and Katz, 1994; Billings and Katz, 1997). Receptivity is dependent upon estradiol, with a preovulatory increase in estradiol resulting in the ovulatory luteinizing hormone (LH) surge (Beach, 1976; Okada et al., 1996).

Sexual Selection in Females

Darwin's theory of sexual selection was developed to account for the evolution of weaponry, ornamentation and other secondary sexual characteristics that are unlikely to contribute to survival, and are commonly more developed in males. However, secondary sexual characteristics are also widespread in females, yet have received little attention from evolutionary biologists (Clutton-Brock, 2009). When females compete for the attention of males, selection may favor the evolution of signals that indicate their fecundity and/or attract the attention of males (Clutton-Brock, 2009). For example, the voices of women become more attractive when they are fertile (Pipitone and Gallup, 2008). Similarly, the mating calls of fertile and infertile macaques differ, and the calls of fertile females are more likely to stimulate ejaculation by their partners and to attract the attention of other males (Pfefferle et al., 2007). Furthermore, female exotic dancers have been shown to earn significantly more in tips from clients during their fertile periods than at other stages of their cycle (Miller et al., 2007). In some species, female size is a good indicator of fecundity: many male insects, fish, reptiles, and amphibians discriminate between females by size when choosing a mate (Cote and Hunte, 1989; Olsson, 1993; Capone, 1995; Verrell, 1995; Gage, 1998; Kraak and Bakker, 1998; Arntzen, 1999).

The tail wagging behavior of female goats in estrus may serve as a visual signal to attract males for mating. Tail wagging may play an important role in the incentive value of the female and therefore sexual motivation in male goats. As previously mentioned, the role of visual stimuli in the incentive value of females has been studied in other species, particularly species in which females have developed pronounced secondary sexual ornaments that attract males, such as primates and birds.

Selection to attract males has probably played an important role in the evolution of female secondary sexual characteristics (Clutton-Brock, 2009). In humans, it is likely that the fat-padded breasts, thighs and buttocks of females may have evolved to attract males (Mealey, 2000; Miller et al., 2007). Sexual selection in females is well-studied in primates, where the evolution and adaptive significance of female sexual swellings have received much attention (reviewed by Nunn, 1999; Stallmann and Froehlich, 2000). Sexually-receptive females display obvious swellings of the perineal region, which reach maximum size around the time of ovulation (Dixon, 1983). Sexual swellings are primarily found in species in which females mate with more than one male during a receptive period (Dixon, 1983; Nunn, 1999). They are also hormone dependent (Dixon, 1983) and attract males for mating independent of olfactory or behavioral cues (Bielert and Anderson, 1985). Tail wagging in goats may function in a similar fashion in that goats are a promiscuous species, tail wagging is hormone dependent, and appears to visually attract male independent of olfactory cues (see Appendix C). However, the benefits of sexual swelling and tail wagging attracting multiple mating partners are presumably different due to the different social structure of these species. In primates, potential benefits include paternity confusion (Hrdy, 1979), thereby lessening the risk of

infanticide, and increased offspring fitness due to increasing the number of potential sires that compete and promoting sperm competition (Harvey and May, 1989). The latter may also occur in goats, since females mate with multiple males and male-male competition for mating is common in this species. Furthermore, in some polygynous ungulates where males compete for females, female-female fighting occurs between females that have entered estrus and need to mate rapidly (Bro-Jorgensen, 2002; Bebie and McElligott, 2006).

Hormonal Regulation of Female Sexual Behavior

Estrus in female goats is stimulated by photoperiod, progesterone pre-treatment, and an elevated concentration of estradiol (Kaplan and Katz, 1994; Billings and Katz, 1997). Estrus in ovariectomized (OVX) goats can be induced by estradiol alone or, outside of the breeding season, by progesterone pre-treatment followed by estradiol treatment (Billings and Katz, 1998). It was first reported that high doses of estradiol (300 µg to 400 µg) can be used to induce estrus in OVX French Alpine goats (Kaplan and Katz, 1994). It was later determined that 30 µg of estradiol is the minimum dose necessary to induce estrus in OVX females (Billings and Katz, 1998).

Aromatizable androgens, in place of estradiol, can be used to induce estrus in female goats, whereas, the non-aromatizable androgen dihydrotestosterone is insufficient to induce estrus (Lindia and Katz, 2000; Imwalle and Katz, 2004b). Imwalle and Katz (2004b) tested the hypothesis that activation of the androgen receptor is required for full expression of female goat sexual behavior. OVX females received progesterone pre-treatment followed by estradiol, testosterone, or sesame oil. Eight hours before or 4 h

after assigned hormone treatment females were treated with either flutamide, an androgen receptor antagonist, or carrier. Treatments with estradiol or testosterone were equally effective in eliciting estrus-typical behaviors compared to treatment with oil. Blocking of the androgen receptor through flutamide treatment reduced receptivity in testosterone-treated, but not estradiol-treated females, suggesting that testosterone at least partly facilitates receptive behaviors as an androgen. Furthermore, flutamide-treatment did not completely inhibit receptivity to levels displayed by oil-treated goats, suggesting that both androgens and estrogens are required for full expression of estrus in female goats. Interestingly, flutamide had a facilitative effect on tail wagging in estradiol-treated females. Imwalle and Katz (2004b), explain that although this observation was unexpected, it is unlikely to be due to flutamide acting as an androgen because oil-treated females given flutamide did not display increases in tail wagging. The researches speculate that antagonism of the androgen receptor may “release a physiologic brake” on the production of the estrogen receptor, therefore making the goats hypersensitive to the effects of exogenous estrogen. They elaborate that Dimitrakakis et al. (2003) treated normal-cycling rhesus monkeys with flutamide or carrier and observed a more than 2-fold increase in estrogen-induced mammary epithelial cell proliferation compared to controls. They also cite that the in vitro blockade of the androgen receptor in prostate cancer cells substantially increases estrogen receptor expression (Kruithof-Dekker et al., 1996).

Male Sexual Behavior

As previously described, sexual behavior can be divided into appetitive and consummatory phases (Beach, 1956). The appetitive phase involves seeking sexual encounters and includes courtship behaviors. A male goat will approach an estrous female in a slightly low-stretched, crouched posture, with his head slightly extended, ears stretched forward, tail straight up, and tongue extended (Geist, 1964; Shank, 1972). The male will then begin courting a female. Courting behaviors include making gobbling calls, ano-genital sniffing, and leg kicking (Geist, 1964; Shank, 1972). Females will respond by courting the male, urinating, or running away from him, but will wag their tails if they are receptive to his courting (Shank, 1972). Females court males by rubbing the neck of the male with their head while he stands with a rigid posture; the male will resume courting once the female stops her rubbing (Shank, 1972). The male's olfactory investigation of a female's expelled urine or perineal region is usually followed by the flehmen lip curl response (Shank, 1972). Flehmen is displayed by most ungulate species including goats, sheep, horses and cattle. Both male and female goats display the behavior, yet it is more common among males (Shank, 1972). Flehmen aids in the transport of fluid-borne substances, such as pheromones, to the vomeronasal organ located at the base of the nasal cavity, facilitating the male's determination of the estrous state of the female (Gelez and Fabre-Nys, 2004). It is believed that female tail wagging functions to fan vaginal odors in the direction of the male, instigating him to continue with the chase or to relay her estrous state to the male (Shank, 1972). Previous work in our lab suggests that tail wagging may also serve to visually attract males from a distance (Imwalle and Katz, 2004a).

The consummatory phase encompasses copulation (Beach, 1956). When a male goat mounts a female he forms a tight foreleg grip around the female's rump which is accompanied by pelvic thrusting. Copulation is usually brief, lasting from a few seconds to a few minutes, which may have evolved to avoid predation. Furthermore, the time from one ejaculation to the next ejaculation is short, so as to provide the male with the ability to mate with as many females as possible in a short period of time (Shank, 1972). When a male goat ejaculates it is accompanied with a powerful thrust in which he throws his head back, and he may go limp and slide off the female (Geist, 1964; Shank, 1972).

A study examining the mating capacity of adult male goats under range conditions in Mexico revealed that there is a strong correlation between the number of females in estrus and the number of times a male ejaculates per day, though a maximum capacity may become apparent if mating load is increased (Mellado et al., 2000). Adult males copulated an average of 9.1 times per day, and females copulated an average of 4 times during an estrus period (Mellado et al., 2000).

Hormonal Regulation of Male Sexual Behavior

Testosterone is responsible for the development and maintenance of male sexual behavior. In ruminants, castration generally decreases the frequency of sexual behaviors. Testosterone replacement restores sexual behavior in sheep (Clegg et al., 1969) and goats (Hart and Jones, 1975). Hart and Jones (1975) found that compared to observations of other species, an unusually high percentage of goats showed long-term retention of sexual activity after castration; however, there was a significant decrease in frequency of ejaculatory responses within one week after castration.

Testosterone is primarily secreted by the Leydig cells of the testes, under the influence of gonadotropins from the anterior pituitary. Specifically, gonadotropin-releasing hormone (GnRH) released from the hypothalamus stimulates the release of LH from the anterior pituitary. Release of LH is episodic; intact bulls experience approximately 5 to 10 LH peaks per 24 hours (Katongole et al., 1971), while intact rams experience 1 to 5 peaks per 24 hours (D'Occhio et al., 1982). Testosterone release is also episodic, occurring 30 min after LH release in intact bulls (Katongole et al., 1971), and mimicking LH peak frequency in both bulls and sheep (Katongole et al., 1971; D'Occhio et al., 1982). Testosterone release in goats is affected by the season, with various goat breeds exhibiting peak testosterone concentrations during their respective breeding seasons (Miyamoto et al., 1987; Grasselli et al., 1992; Perez-Llano and Mateos-Rex, 1994).

Table 1

| | |
|-------------------|--|
| Head Butting | A head-to-head strike or a strike to any part of another goat's body; can occur with or without a goat rearing-up on its hind legs. The contact can range from a soft nudge to an audible crashing of heads. |
| Ano-genital Sniff | One goat sniffs the ano-genital area of another goat. |
| Urination | Adult males will urinate on their own faces and beards with an extended penis (self-enurination). Females will urinate in response to an ano-genital sniff. |
| Flehmen | A goat stands open-mouthed and curls its upper lip exposing the gums, generally with its head and neck extended. Usually occurs after sniffing the urine or the genitalia of oneself or another goat. |
| Courting | A goat takes prancing steps towards another goat with its tongue sticking out, head extended, and making gobbling vocalizations. |
| Leg Kick | A courtship behavior in which one goat strikes at another with its front leg, usually during or after ano-genital sniffing. |
| Tail Wagging | A female goat in estrus wags her tail from side to side. Can occur with or without stimulation from another goat, such as an ano-genital sniff. |
| Mounting | One goat places its front legs and upper torso on another in any orientation. May or may not include pelvic thrusting. |

Table 1. Behaviors commonly displayed by male and female goats in a social or sexual context

CHAPTER 2

Female tail wagging elicits partner preference in male goats

Abstract

We hypothesized that the tail wagging behavior of female goats in estrus contributes to their incentive value, thus affecting partner preference in males. Partner preference was tested by allowing males to choose between two females located in pens at opposite ends of an arena. Females were non-estrous, estrous, or flutamide-treated estrous. Results from a previous study in our laboratory showed that flutamide treatment enhanced tail wagging in estrous females, and its use would ensure that females display high levels of tail wagging. Thirteen males were individually tested in six 5-min trials. Preference scores (PS) were calculated from time spent with each female, and number of visits to each female was recorded. Tail wagging by females was recorded. Flutamide-treated estrous females spent more time tail wagging and displayed more bouts of tail wagging than other females. Males showed no preference for an estrous versus a non-estrous female (PS 46% vs. 54%, respectively). Males preferred a flutamide-treated estrous female more than an estrous female (PS 64% vs. 36%, respectively) or a non-estrous female (PS 68% vs. 32% for a flutamide-treated estrous and a non-estrous female, respectively), and visited the flutamide-treated estrous female the most. Data grouped by bouts of tail wagging or time tail wagging showed that males preferred females that displayed more bouts of tail wagging or spent more time tail wagging. Tail wagging by females initiated male approach behaviors and maintained the attention of the males towards the females, indicating that tail wagging serves as both attractivity and

proceptivity. We conclude that tail wagging is an important attractive and proceptive behavior affecting partner preference in the male goat.

Introduction

Sexual motivation is stimulated by the presence of a suitable mate (Beach, 1956). Approach behaviors by the male are activated by appropriate incentives of the female, therefore sexual motivation is an incentive motivation (Agmo, 1999). Attractivity describes the female's ability to stimulate interest from a male and is typically measured by observing the behavior of the male, such as approaching and investigating the female (Beach, 1976). Proceptivity is the sum of sexual behaviors exhibited by the female towards the male in order to initiate or maintain sexual interaction with the male and is typically measured by observing the behavior of the female, such as the ram-seeking behavior of ewes (Beach, 1976). Female goats in estrus engage in a behavior known as tail wagging, which is thought to aid in male determination of estrous state by wafting pheromones (Shank, 1972). Results from a previous study in our laboratory suggest that tail wagging may also serve to visually attract males from a distance. The study examined the role of androgens in the expression of female sexual behavior, and showed that with progesterone-priming, estradiol and testosterone are equally effective in eliciting estrus-typical behavior in ovariectomized females (Imwalle and Katz, 2004b). However, it remains unclear whether androgens influence estrous behaviors alone or in some combination with estrogen. This study also revealed that flutamide, an androgen receptor antagonist, enhanced tail wagging in estrous females (Imwalle and Katz, 2004b). Tail

wagging may play an important role in the incentive value of the female goat and therefore sexual motivation in males. The current study was designed to measure the effect of female tail wagging on male partner preference, an indirect measure of sexual motivation, using flutamide-treated estrous females to ensure that females display high levels of tail wagging. A partner preference test simultaneously exposes the subject to two socially-relevant stimuli and the time spent in proximity of each stimulus is a measure of preference for that stimulus. We predicted that sexually experienced male goats will prefer a female that tail wags more frequently.

Materials and Methods

Animals and treatments

All animals were sexually experienced French Alpine goats, and were group-housed by sex in an open barn with natural lighting and free access to an outdoor field. The subjects were 13 males; previously ovariectomized adult females ($n = 10$) were used as both the sexually receptive and non-receptive stimuli. Estrus was induced in ovariectomized females with sc injections of progesterone 72 h (10 mg/goat) and 48 h (5 mg/goat) prior to estradiol (100 µg/goat) (Steraloids Inc., Newport, RI), as previously described by Billings and Katz (1997). Flutamide (Sigma-Aldrich, St. Louis, MO) was dissolved in DMSO at a concentration of 200 mg/ml. Females were treated with flutamide (9 mg/kg, sc) or vehicle 8 h before and 4 h after the estradiol injection (Imwalle and Katz, 2004b). Preference testing began 14 h after estradiol treatment. Estrus detection was conducted 1 h before the start of testing using a sexually experienced male (not

serving as a subject). The male was able to interact with the females but was on a lead to prevent him from mounting the females. The female's receptivity was observed to determine if she was in estrus.

All subjects were acclimated to the testing apparatus in multiple 5 min sessions over a 2 day period 1 week prior to the start of the experiment. Subjects were first exposed to the apparatus in groups. The size of the group was reduced until the subject was alone. A subject was considered acclimated to the apparatus once it could remain in the apparatus alone for 5 min with no apparent signs of distress, such as frantic running and vocalizing or trying to escape.

Test apparatus

Small, wire pens (1.5 m x 1.5 m) were located at opposite ends of a test arena (17.6 m x 3.6 m) located in an enclosed barn (Figure 1). The pens allowed for visual and olfactory investigation and limited physical contact between the male and female. A 7.6 m incentive zone was defined in front of each small pen, with a 2.4 m neutral zone located between the incentive zones. Each incentive zone was partially partitioned off from the neutral zone using shade cloth that extended from floor to ceiling. This prevented the male from being able to see both females at the same time while in the neutral zone. In a previous study in which no partitions were used (see Appendix C), males stayed in the neutral zone looking back and forth between females, thereby not making a choice.

Partner preference testing

Males were individually tested for 5 min for partner preference by having them choose between a single female from two of the three treatments (non-estrous, estrous, or flutamide-treated estrous). At the start of each test the male was led into the neutral zone and shown each female and then released from the center of the neutral zone (Figure 1). Time in each incentive zone and number of visits to each incentive zone was recorded. The male was considered in or out of the incentive zone once his front legs crossed the vertical plane of the incentive zone. Bouts of tail wagging and time tail wagging were recorded for each female. A bout of tail wagging was defined as anytime the female was continuously wagging her tail. A bout began when the female started wagging her tail and ended when she stopped wagging her tail. A new bout began when the female resumed wagging her tail. An individual bout of tail wagging is usually characterized by an uptick of the tail at the start of the wagging motion. Time tail wagging was the total time that the female was wagging her tail.

Three trials were conducted so that every female treatment pair was tested. This was also replicated using a switchback design to eliminate side bias. A total of six trials were conducted twice a week for 3 weeks with the pairings presented in a random order.

Statistical analysis

Bouts of tail wagging and time tail wagging were compared using single factor ANOVA. Comparisons were also made based on treatment using single factor ANOVA. Post-hoc differences were determined using Duncan's Multiple Comparison Test ($P < 0.05$). A preference score (PS) was calculated for each incentive zone: $PS = (\text{time in one}$

incentive zone / time in both incentive zones) x 100. Mean preference scores were compared with the Mann-Whitney U test ($P < 0.05$), and mean numbers of visits to each incentive zone were compared using a paired t-test ($P < 0.05$). Because means were used, data from males that did not make any choice between females for one of the two trials of a treatment pair were not used. Preference scores between low or high tail wagging females independent of treatment were also compared. Data were grouped by time tail wagging or bouts of tail wagging based on two standard errors and analyzed using a t-test ($P < 0.05$). Two standard errors below the mean was considered low and all other instances were considered high. Statistical analysis was performed using NCSSTM software (NCSS Statistical Software, 2001, Kaysville, UT).

Results

There was a significant effect of treatment on bouts of tail wagging and time tail wagging for females ($F_{2,156} = 34.35$ and $F_{2,156} = 20.71$, respectively; $P < 0.001$). Flutamide-treated estrous females had more bouts of tail wagging and spent more time tail wagging ($P < 0.001$; Figure 2).

Males showed no preference for an estrous versus a non-estrous female ($P > 0.05$; Figure 3). Males preferred a flutamide-treated estrous female over an estrous female ($P < 0.01$) and made more visits to the flutamide-treated estrous female incentive zone ($P < 0.05$). Males preferred a flutamide-treated estrous female over a non-estrous female ($P < 0.001$) and made more visits to the flutamide-treated estrous female incentive zone ($P < 0.01$). Data from males that spent their entire choice time with one female, therefore not

choosing between the two females, for one of the two trials of a treatment pair were not used. This occurred four times and did not affect the significance of any of the results. In fact, the four males' preferences coincided with the hypothesis. When data were grouped by bouts of tail wagging or time tail wagging based on two mean standard errors, males preferred a female with high bouts of tail wagging compared to a female with low bouts ($P < 0.001$; Figure 4). Similarly, males preferred a female which spent more time tail wagging compared to one which spent less time tail wagging ($P < 0.001$).

Discussion

Flutamide treatment enhanced tail wagging in estrous females, as it did in a previous study in our laboratory (Imwalle and Katz, 2004b). This effect may be due to an increase in estrogen receptors, which has been reported in various tissues after the administration of flutamide, such as the heart, T lymphocytes and the gubernaculum (Samy et al., 2000; Yang et al., 2002; Hsieh et al., 2006). An increase in estrogen receptors may make female goats hypersensitive to the effects of exogenous estradiol; however the target tissue of this effect is unknown.

The incentive properties of tail wagging initiated approach behaviors in the male and maintained sexual interaction with the male, as males made more visits to and spent more time with the flutamide-treated estrous female than either the estrous or non-estrous female. Therefore by Beach's (1976) definitions, when tail wagging initiates approach behaviors from a male it can be thought of as attractivity, and when the female continues

to display the behavior to maintain interactions with the male it can be thought of as proceptivity.

Males showed a preference for tail wagging and not estrous state. It is unclear if this preference is due to the visual cue of tail wagging or from the possible wafting of pheromones caused by tail wagging, or from a combination of both. However, it appears that visual cues may be more important to a male's ability to identify estrous females than olfactory cues, since there was no preference between estrous and non-estrous females whose tail wagging behavior was not different. It is presumed that the main difference between these females is olfactory cues. The lack of difference between the amount of tail wagging displayed by estrous and non-estrous females is most likely due to the use of single females. After this study concluded, it was observed that in the presence of a male, single females tend to tail wag less than females in a group. There is also individual variation among females with respect to how much they tail wag. Moreover, previous studies were unable to determine male partner preference between a single estrous and non-estrous female using a straight-arm runway, Y-maze or a different partner preference test (see Appendices A-C, respectively). During the partner preference study it was observed, but not quantified, that the estrous females displayed variable levels of tail wagging during different trials, and that males preferred the high tail wagging females. This prompted the design of the current study in which female tail wagging could be enhanced with flutamide.

Similarly, sexually experienced bulls tested for preference between an estrous and non-estrous heifer using a test pen did not display a preference (Geary et al., 1991). However, bulls did prefer a pair of heifers engaging in female-female mounting, a visual

cue of estrus, over non-estrous females and estrous females unable to mount one another (Geary and Reeves, 1992). Cattle and goats live in herds in which females form groups in the center of the home range and adult males live on the periphery (Crook, 1969; Geist, 1964; Kilgour et al., 1977). When in estrus, female goats and cattle form sexually active groups that engage in female-female mounting, as well as other behaviors (Blockey, 1978; Kilgour et al., 1977; Shank, 1972). Visual stimuli may be more important in locating estrous females as olfactory cues may not travel great distances across a home range. Furthermore, sexual performance of male goats is enhanced when males view females mounting one another before a sexual performance test (Shearer and Katz, 2006). Enhancement of sexual performance also occurs in male goats and cattle when they are allowed to watch conspecifics mating (Mader and Price, 1984; Price et al., 1984). These effects may have resulted from selective pressures to identify estrous females from a distance. Partner preference tests that use groups of females rather than single females may be better suited for the goat.

The current study only tested partner preference of sexually experienced males because we did not have a large enough group of sexually naive males. It would be useful to test the partner preference of young, sexually naive males because current methods of measuring sexual performance (serving capacity) in young goats can be inaccurate in predicting future sexual performance. Juvenile sexually naive goats display separation anxiety when removed from pen mates and require up to six sexual interactions with receptive females before attaining a consistent serving capacity (Imwalle and Katz, 2004a). Sexual motivation in young males may be a better predictor of adult performance, particularly if brain systems underlying motivation develop earlier than

physical systems mature. Identifying replacement breeders when males are still young would increase the efficiency and productivity of breeding programs.

In conclusion, sexually experienced male goats display a preference for females based on the amount of female tail wagging. Tail wagging can be classified as attractivity and proceptivity, depending on the context of the behavior and whether female or male behavior is the focus of the study. Tail wagging is an important attractive and proceptive behavior affecting partner preference in the male goat.

Figure 1

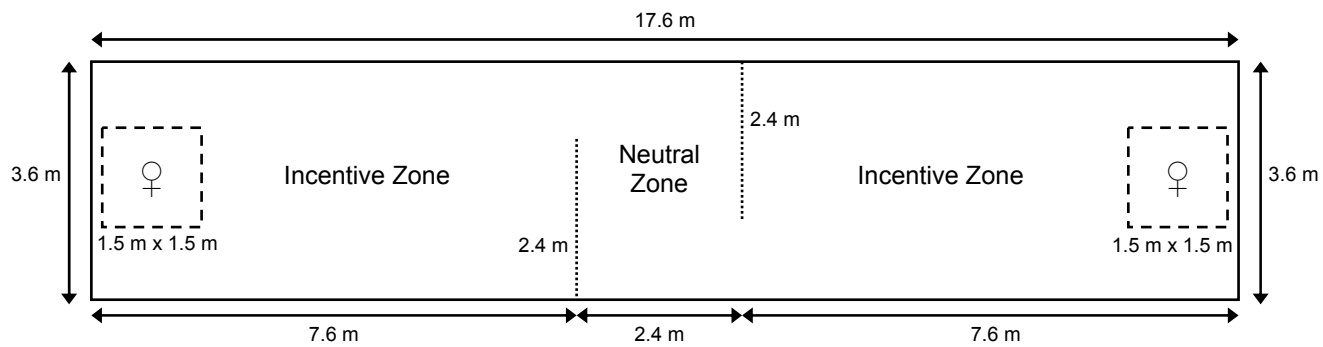


Figure 1. Test apparatus. Dotted lines indicate shade cloth and dashed lines indicate wire fence pens. Each pen contained a single female. A single male was released from the center of the neutral zone at the start of each test. The neutral zone was designed so that the male could not see both stimuli at the same time.

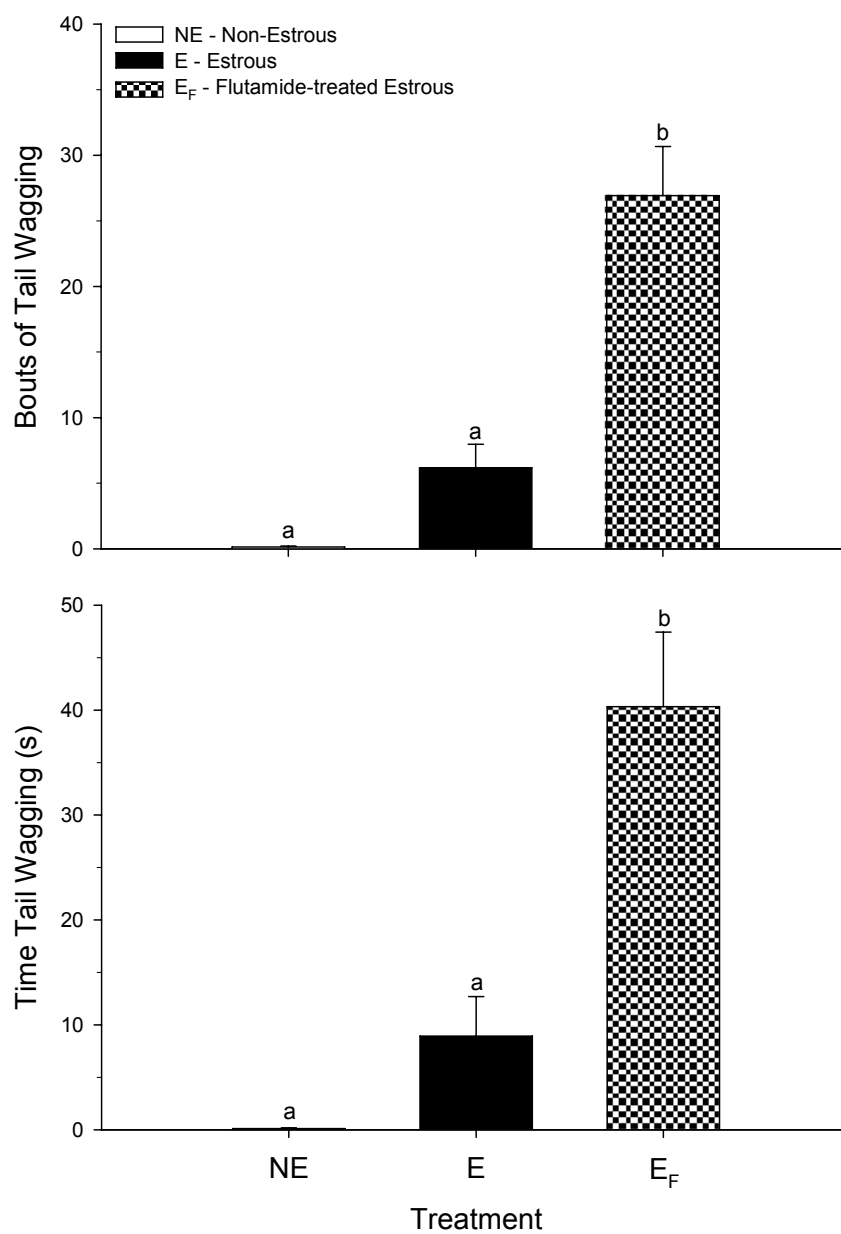
Figure 2

Figure 2. Mean (\pm SEM) bouts of and time tail wagging for all treatments. Bars with different superscripts differ (Duncan's, $P < 0.05$).

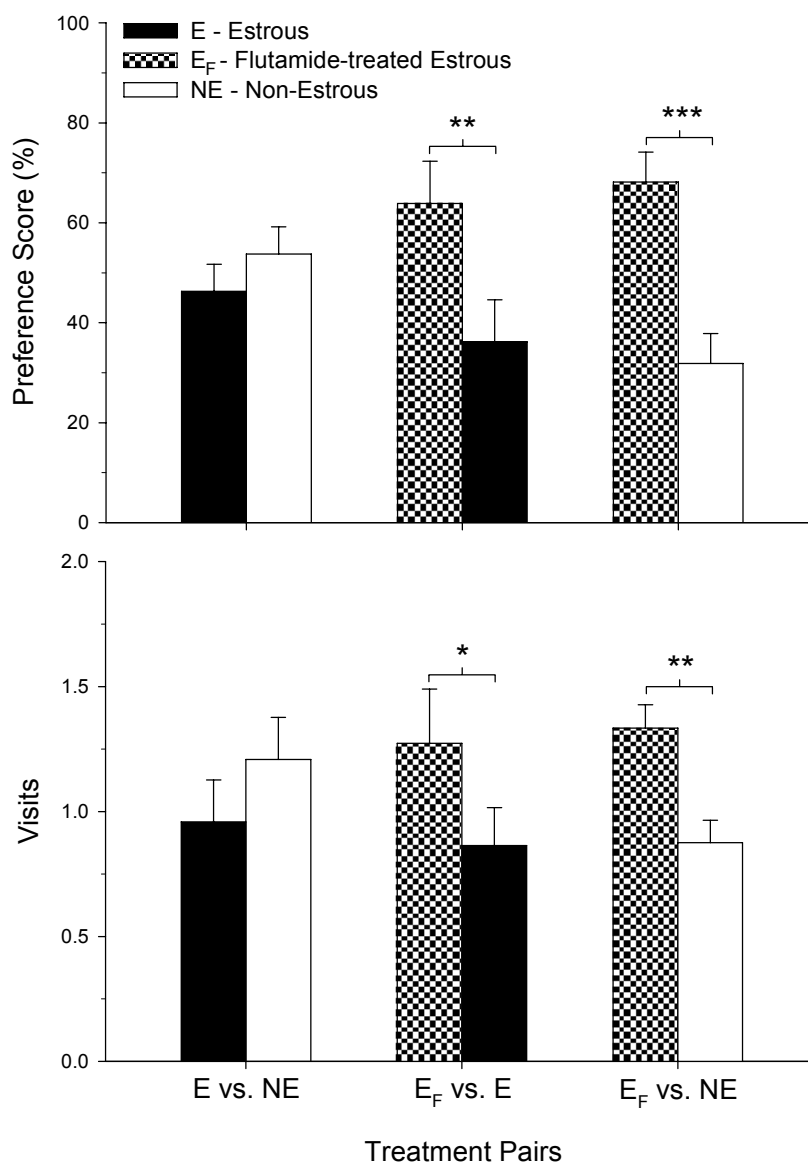
Figure 3

Figure 3. Mean (\pm SEP) preference scores and mean (\pm SEM) visits by female treatment pairs. Bars with superscripts differ (Mann-Whitney U test, ** = $P < 0.01$, *** = $P < 0.001$; paired t -test, * = $P < 0.05$, ** = $P < 0.01$).

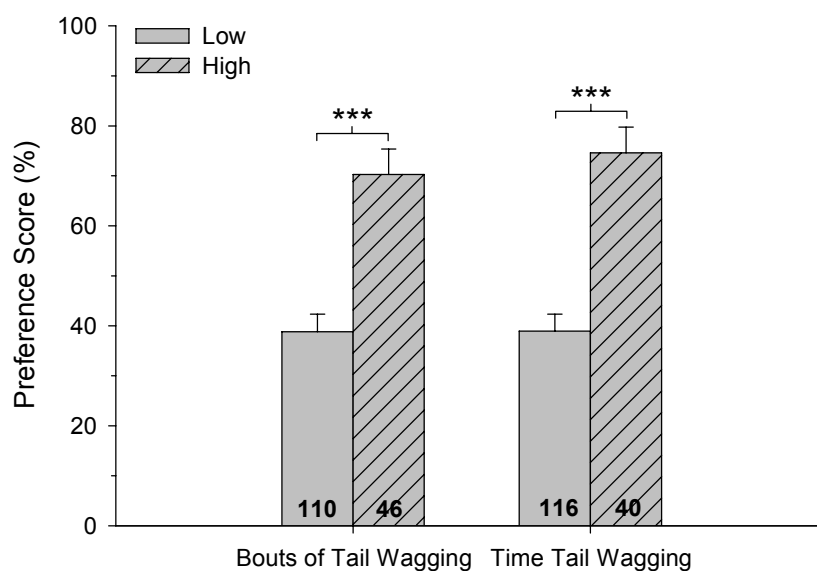
Figure 4

Figure 4. Mean (\pm SEM) preference scores for all trials grouped by time tail wagging or by bouts of tail wagging. Bars with superscripts differ (t-test, $** = P < 0.01$, $*** = P < 0.001$). Numbers inside bars indicate number of trials.

CHAPTER 3

Female tail wagging enhances sexual performance in male goats

Abstract

Preference testing has shown that sexually experienced male goats choose females that are tail wagging, a behavior that may function as both attractivity and proceptivity, over those that are not. We hypothesized that exposure to females expressing high rates of tail wagging would arouse males, increasing sexual performance. Tail wagging rate could be manipulated because we have shown previously that flutamide treatment increases the frequency of tail wagging in estrous goats. Sexually experienced males observed different stimuli for 10 min before a 20 min sexual performance test (SPT). The stimuli were an empty pen (MT), or groups of three females that were all estrous (E), non-estrous (NE), estrous+flutamide (E_F) or non-estrous+flutamide (NE_F). During the stimulus observation period, tail wagging was recorded. During SPT, frequencies and latencies of sexual behaviors were recorded. E_F females displayed the most tail wagging. Viewing E_F females before SPT increased the number of ejaculations attained by males and decreased the latencies to first and second ejaculation, as well as the inter-ejaculatory interval. Viewing estrous females (E and E_F) before SPT decreased the latency to first mount, as compared to non-estrous females (NE and NE_F). We conclude that male goats are sexually aroused by tail wagging. This study and previous work demonstrate that tail wagging functions as both attractivity and proceptivity in goats.

Introduction

Beach (1976) defined three components of female sexual behavior – attractivity, receptivity and proceptivity. Attractivity is the female's ability to stimulate interest from a male, and is typically measured by observing the behavior of the male, such as approaching and investigating the female. Proceptivity is any behavior exhibited by the female that initiates or maintains sexual interaction with the male. Female goats in estrus engage in a behavior known as tail wagging. Preference testing has shown that sexually experienced males make more visits to and spend more time with females that tail wag frequently (Chapter 2). Therefore, tail wagging functions as both attractivity and proceptivity.

Goats are herd animals. Females and young live in the center of the home range, while adult males live on the periphery (Crook, 1969; Geist, 1964). When in estrus, female goats form sexually active groups that display courtship behaviors, such as tail wagging and female-female mounting (Shank, 1972). The sexual performance of male goats is enhanced when males view females mounting one another before a sexual performance test (Shearer and Katz, 2006). Enhancement of sexual performance also occurs in male goats when they are allowed to observe males mounting females prior to a sexual performance test (Price et al., 1984). Tail wagging and female-female mounting may play a key role in males locating estrous females from a distance, as olfactory cues may not travel great distances across a home range. We hypothesize that exposure to females expressing high rates of tail wagging will arouse males, thus increasing their sexual performance. Results from previous studies in our laboratory showed that

flutamide, an androgen receptor antagonist, increases the frequency of tail wagging in estrous females (Imwalle and Katz, 2004b; Chapter 2). The current experiment was designed to measure the effect of female tail wagging on male sexual performance using flutamide-treated females.

Materials and Methods

Animals and treatments

The research protocol was approved by the Rutgers University Animal Care and Facilities Committee. All animals were sexually experienced French Alpine goats. Males ($n = 7$) were used as the subject animals, and ovariectomized females ($n = 20$) were used as the stimulus animals. Animals were group-housed by sex in an open barn with natural lighting and free access to an outdoor field.

Estrus was induced in ovariectomized females with melengestrol acetate (0.5 mg, p.o.) 72 h and 48 h prior to estradiol injection (100 μ g, s.c.) (Steraloids Inc., Newport, RI). Flutamide (Sigma-Aldrich, St. Louis, MO) was dissolved in 3:1 benzyl benzoate:DMSO at a concentration of 200 mg/ml. Females were treated with flutamide (9 mg/kg, s.c.) or vehicle 8 h before and 4 h after the estradiol injection (Imwalle and Katz, 2004b). Seven females were randomly chosen to receive flutamide treatment throughout the experiment. Behavior tests were conducted 14 h after estradiol treatment. The females' receptivity was observed to detect estrus 1 h before the start of testing using a sexually experienced male (not a subject male). The male was able to interact with the females but was on a lead to prevent him from mounting the females.

Behavior tests

The behavior tests were conducted in a 3.5 m x 9 m test arena located in an enclosed barn. A 2 m x 3 m wire-panel pen was located in one corner of the test arena. Males were individually tested in five 30-min behavior tests consisting of a 10-min stimulus observation period (SOP) followed by a 20-min sexual performance test (SPT). Females were randomly assigned to be used as stimulus females or SPT females throughout the entire experiment.

During the SOP, a male located in the test arena was able to observe different social and sexual stimuli located in the small pen. The pen allowed for visual and olfactory investigation and limited physical contact. The stimulus conditions were an empty pen (MT), or groups of three females that were all estrous (E), non-estrous (NE), estrous+flutamide (E_F) or non-estrous+flutamide (NE_F). Bouts of tail wagging were recorded for each female. A bout of tail wagging was defined as anytime the female was continuously wagging her tail. A bout began when the female started wagging her tail and ended when she stopped wagging her tail. A new bout began when the female resumed wagging her tail. An individual bout of tail wagging is usually characterized by an uptick of the tail at the start of the wagging motion. After 10 min, the male was restrained while the females, if present, were removed. For the SPT, an estrous female was released into the test arena containing the male. Frequencies and latencies of sexual behaviors were recorded. Males were tested once for each of the 5 stimulus conditions in a random order. Tests were conducted once a week for 5 wk. On each test day all 5

stimulus conditions were being tested. On each test day, after males were tested they were kept separate from males that had yet to be tested.

Statistical analysis

Bouts of tail wagging were compared using single factor ANOVA. Mount and ejaculation frequencies and latencies were compared using repeated measures ANOVA. Post-hoc differences were determined using Fisher's LSD multiple comparison test ($P < 0.05$). Latencies were transformed using the $\log(x+1)$ to normalize the data set. Statistical analysis was performed using NCSSTM software (NCSS Statistical Software, 2001, Kaysville, UT).

Results

Estrous females treated with flutamide (E_F) displayed more bouts of tail wagging than all other females ($P < 0.001$; Table 2), supporting our previous findings. Viewing females that tail wagged more (E_F) prior to a sexual performance test increased the number of ejaculations attained by males ($P < 0.01$; Figure 5). Table 3 displays the untransformed latencies to sexual behaviors. The latency to first and second ejaculation ($P < 0.05$ and $P < 0.01$, respectively; Figure 6) and the inter-ejaculatory interval ($P < 0.01$; Figure 7) were shortest for males exposed to females that tail wagged more (E_F). The latency to first mount was shorter for males exposed to estrous females (E and E_F) than non-estrous females (NE and NE_F) ($P < 0.05$; Figure 8). The difference from the mean for each latency measure was summed (Figure 9). Latency measures included time

to first mount, time to first and second ejaculation, and inter-ejaculatory interval. The lesser the cumulative score, the faster males engaged in sexual activities. Males exposed to females that tail wagged more (E_F) engaged in sexual activities more rapidly, indicating that they were more aroused.

Discussion

Females may use the visual cues of tail wagging and female-female mounting to communicate their estrous state to distant males, as olfactory cues may not travel across a home range. Sexual performance of male goats is enhanced when males view groups of females engaging in female-female mounting (Shearer and Katz, 2006) or with artificially enhanced tail wagging (E_F) prior to a sexual performance test. Flutamide treatment produced an almost four-fold increase in tail wagging among estrous females. The sexual performance of males exposed to a group of non-flutamide-treated estrous females was not significantly enhanced. Shearer and Katz (2006) report similar results, in which males exposed to mounting animals (male or female) showed enhanced sexual performance compared to males exposed to non-mounting estrous females. Tail wagging was not recorded in that study, but it is presumed that the estrous females used would display similar tail wagging levels to the estrous females used in the current study. Moreover, in a partner preference test males demonstrated no preference for an estrous versus a non-estrous female, but preferred a flutamide-treated estrous female over an estrous or non-estrous female (Chapter 2). In that study, flutamide-treated estrous females tail wagged more than estrous or non-estrous females. It appears there is a tail wagging threshold at

which males will display partner preference or an increase in sexual performance. It is unlikely that flutamide-treatment alone would account for these effects, as males showed no increase in sexual performance in response to non-estrous females treated with flutamide.

These effects may have resulted from selective pressures to identify estrous females from a distance. Males that can identify and be aroused by estrous females would have an advantage over other males, as they would be able to locate and mate with estrous females sooner and more frequently. They may also sire more offspring, because by the time less responsive males have located the estrous females, the females' receptivity may have ended due to repeated copulations with other males (Price et al., 1998). A female's reproductive fitness is most likely affected by tail wagging as well. There are individual variations in the amount of tail wagging that estrous females display. Females that tail wag more may have a better chance of being impregnated if tail wagging is able to attract and arouse males.

Tail wagging may not only function to attract males from a distance, as female goats continue to tail wag in the presence of a male. Flehmen is a behavior displayed by most ungulate species including goats, sheep, horses and cattle. Both males and females display the behavior, yet it is more common among males. It is thought that flehmen aids in the transport of fluid-borne substances, such as pheromones, to the vomeronasal organ so the male can determine the estrous state of the female (Gelez and Fabre-Nys, 2004). It is believed that female tail wagging functions to fan vaginal odors in the direction of the male, to relay her estrous state to the male or instigating him to continue to pursue the female (Shank, 1972). Female goats respond to a courting male by urinating, courting the

male, or running away from the male while tail wagging (Shank, 1972). Olfactory investigation of a female's expelled urine or perineal region is usually followed by the flehmen lip curl response (Shank, 1972). Female goats will repeatedly approach the male, tail wag, move away and then re-approach the male. Females repeat this sequence until the male pursues her or until he performs a sexual behavior. The approach-withdrawal pattern may function to correctly orient the male in a posterior position to allow him to correctly mount the female (Beach, 1976). Females continue to wag their tails as a male mounts (personal observation), which may help to stimulate and guide the penis for intromission.

In conclusion, male goats are sexually aroused by female tail wagging. Results from this study and previous work demonstrate that tail wagging functions as both attractivity and proceptivity in goats.

Table 2

Mean (\pm SEM) bouts of tail wagging for three females during the seven 10-min stimulus observation periods / stimulus condition

| Stimulus condition | NE | NE _F | E | E _F |
|-----------------------|-------------|-----------------|-------------|-----------------|
| Bouts of tail wagging | 17 \pm 10 | 24 \pm 14 | 61 \pm 27 | 223 \pm 51*** |

*** $P < 0.001$

NE – non-estrous; NE_F – non-estrous treated with flutamide; E – estrous;
E_F – estrous treated with flutamide

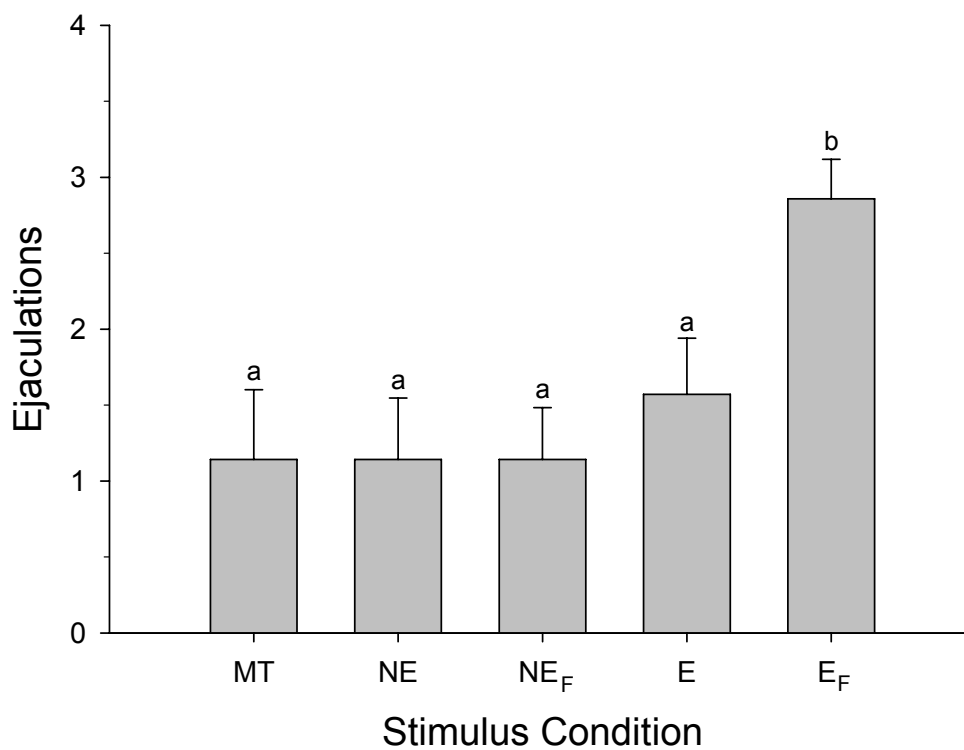
Figure 5

Figure 5. Mean (\pm SEM) ejaculations during the 20-min sexual performance test for each stimulus condition. Bars with different superscripts differ. MT – empty; NE – three non-estrous females; NE_F – three non-estrous females treated with flutamide; E – three estrous females; E_F – three estrous females treated with flutamide.

Table 3

Mean (\pm SEM) latencies of sexual behaviors in seconds for subject males

| Stimulus condition | Latency to first mount | Latency to first ejaculation | Latency to second ejaculation | Inter-ejaculatory interval |
|--------------------|------------------------|------------------------------|-------------------------------|----------------------------|
| Empty | 25 \pm 4 | 634 \pm 205 | 1009 \pm 92 | 490 \pm 47 |
| NE | 201 \pm 167 | 469 \pm 200 | 841 \pm 169 | 270 \pm 29 |
| NE _F | 189 \pm 168 | 417 \pm 206 | 927 \pm 138 | 509 \pm 118 |
| E | 13 \pm 5 | 388 \pm 193 | 833 \pm 149 | 470 \pm 112 |
| E _F | 9 \pm 3 | 56 \pm 15 | 367 \pm 95 | 311 \pm 86 |

NE – non-estrous; NE_F – non-estrous treated with flutamide; E – estrous;

E_F – estrous treated with flutamide

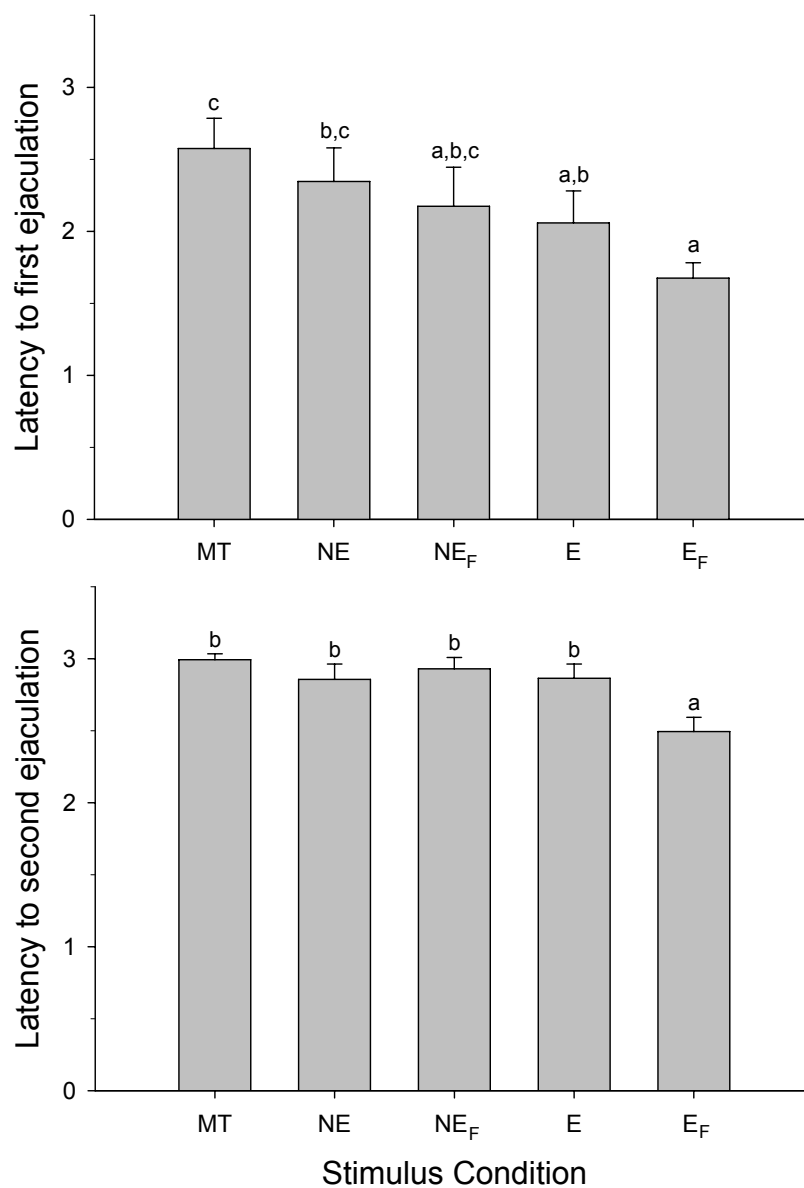
Figure 6

Figure 6. Mean (\pm SEM) $\log(x+1)$ transformed latencies to first and second ejaculation during the 20-min sexual performance test for each stimulus condition. Bars with different superscripts differ. MT – empty; NE – three non-estrous females; NE_F – three non-estrous females treated with flutamide; E – three estrous females; E_F – three estrous females treated with flutamide.

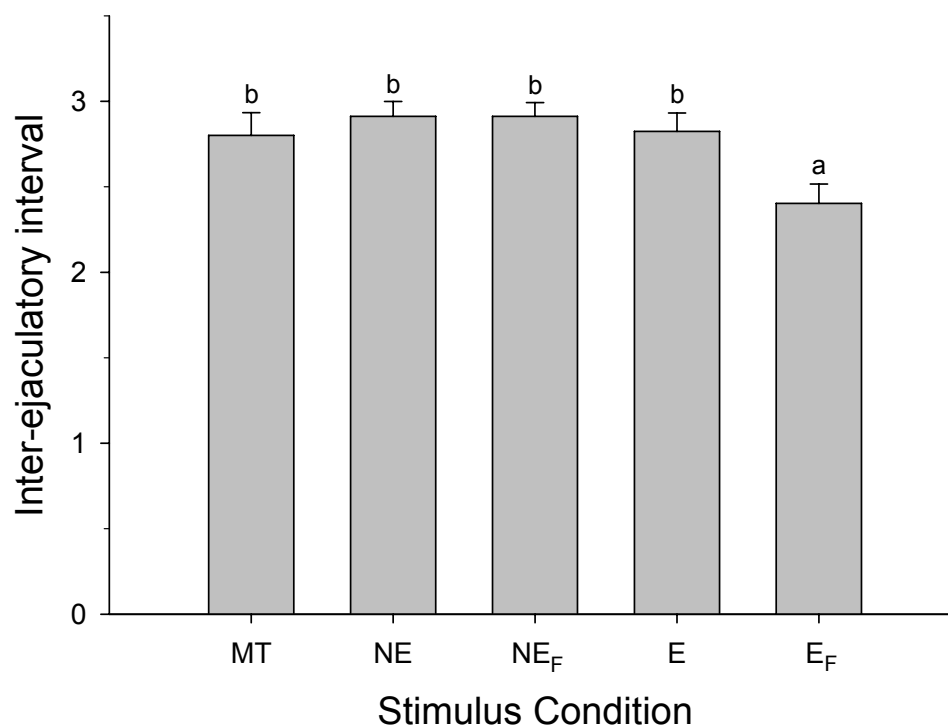
Figure 7

Figure 7. Mean (\pm SEM) $\log(x+1)$ transformed inter-ejaculatory interval during the 20-min sexual performance test for each stimulus condition. Bars with different superscripts differ. MT – empty; NE – three non-estrous females; NE_F – three non-estrous females treated with flutamide; E – three estrous females; E_F – three estrous females treated with flutamide.

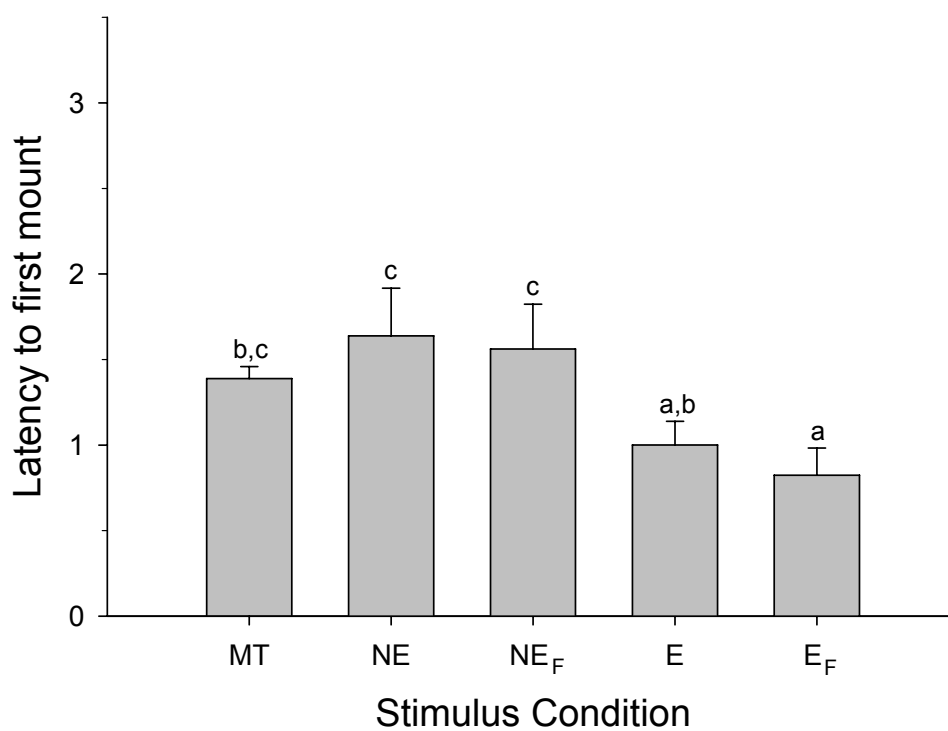
Figure 8

Figure 8. Mean (\pm SEM) $\log(x+1)$ transformed latency to first mount during the 20-min sexual performance test for each stimulus condition. Bars with different superscripts differ. MT – empty; NE – three non-estrous females; NE_F – three non-estrous females treated with flutamide; E – three estrous females; E_F – three estrous females treated with flutamide.

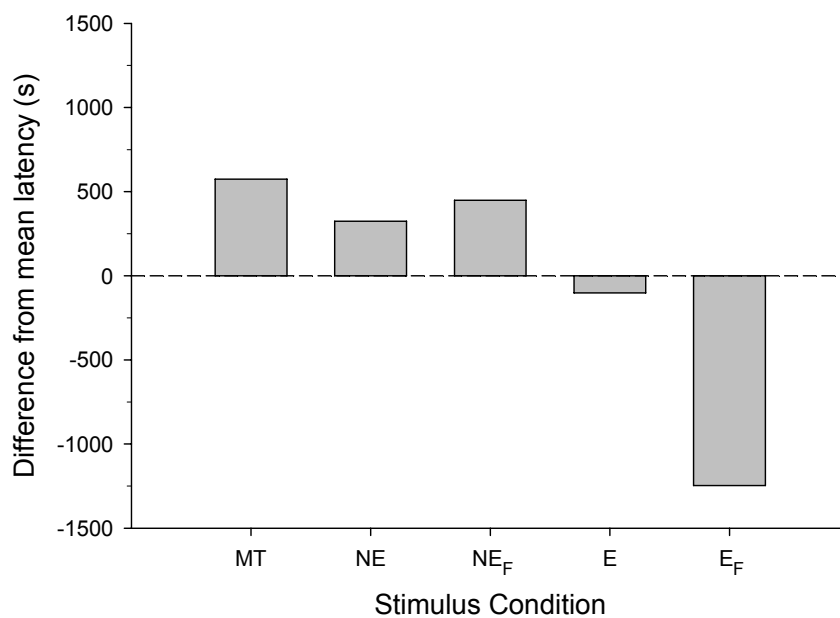
Figure 9

Figure 9. Cumulative latency scores. For each stimulus condition, the differences from the mean for each latency were summed. MT – empty; NE – three non-estrous females; NE_F – three non-estrous females treated with flutamide; E – three estrous females; E_F – three estrous females treated with flutamide.

CHAPTER 4

Partner preference and sexual performance in male goats

Abstract

Sexual performance is a combination of the physical ability to mate and sexual motivation. An indirect measure of sexual motivation is strength of partner preference, because sexual motivation can be stimulated by the presence of suitable mates. This study was designed to 1) determine if partner preference in male goats is dependent on hormonal state or sexual experience of the male, and 2) to assess the relationship between partner preference and sexual performance in sexually experienced and sexually naïve males. Males were individually tested in two 5-min trials for partner preference between groups of estrous (E) or non-estrous (NE) females located in pens at opposite ends of an 18 m runway. Preference scores (PS) were calculated from time spent within 1 m of each pen. In Experiment 1, sexually experienced, intact males (n=17) and sexually naïve, castrated males (n=11) were tested. Nine castrates were retested during a period of testosterone propionate (TP) treatment. Also, the sexually experienced bucks were administered three sexual performance tests. In Experiment 2, sexually naïve, intact males (n=18) were tested for preference, administered six sexual performance tests, and then retested for preference. Sexually naïve castrates showed no preference (PS=48% and 52% for E and NE females, respectively), whereas sexually naïve, TP-treated castrates and sexually experienced, intact males preferred E females (PS=69% and 66%, respectively, for E females). Sexually naïve, intact males preferred E females before and after sexual experience (PS=65% and 58%, respectively, for E females). There were no

correlations between sexual performance and PS for sexually experienced or sexually naive males. We conclude that preference for estrous females is dependent on hormonal state, not sexual experience, in male goats. Furthermore, partner preference is not a reliable indicator of sexual performance in male goats.

Introduction

Goats live in herds, in which females form groups in the center of the home range and adult males live on the periphery (Crook, 1969; Geist, 1964). When in estrus, female goats form sexually active groups that display courtship behaviors, such as tail wagging and female-female mounting (Shank, 1972). Females may use these visual cues to communicate their estrous state to distant males. Previous studies have shown that the sexual performance of male goats is enhanced when males view groups of estrous females tail wagging (Chapter 3) or engaging in female-female mounting (Shearer and Katz, 2006) prior to a sexual performance test. Males also prefer estrous females that tail wag frequently in partner preference tests (Chapter 2). The current study is designed to further explore the relationship between male partner preference and female-female mounting and tail wagging. A partner preference test was designed so that males could observe two different groups of females from a distance, simulating the goat's natural environment. Also, to determine if partner preference in male goats is dependent on hormonal state or sexual experience of the male, sexually experienced intact males, sexually naive intact males, sexually naive castrates, and sexually naive castrates treated

with testosterone propionate were tested. We predicted that partner preference in male goats is dependent on hormonal state and not sexual experience.

Furthermore, understanding what motivates a male goat to engage in sexual activity could increase the efficiency and productivity of goat breeding programs. Partner preference can serve as an indirect measure of sexual motivation, which is one component of sexual performance. Sexual performance is the combination of competent physical abilities and sexual motivation. High sexual performance requires both high motivation and adequate physical abilities, but low sexual performance does not necessarily indicate low motivation (Beach, 1956; Katz and McDonald, 1992). Current methods of measuring sexual performance in goats (serving capacity) can be inaccurate in predicting future sexual performance, especially in young males. This may be due to physical and psychological factors that affect sexual behavior developing at different rates. Sexual motivation may be well-developed early, but inadequate size and lack of strength, endurance and coordination may impair performance. Research in sheep and rats suggests that motivation may develop early. Sexually naive male rats and sheep both show interest in estrous females before ever mating (Lopez et al., 1999; Price et al., 1992). Additionally, it has been shown that juvenile sexually naive goats display separation anxiety when removed from pen mates and require up to six sexual interactions with receptive females before attaining a consistent serving capacity (Imwalle and Katz, 2004a). Sexual motivation in young males may be a better predictor of adult performance, particularly if brain systems underlying motivation develop earlier than physical systems mature. Identifying replacement breeders when males are still young would increase the efficiency and productivity of breeding programs. The sexually

experienced and sexually naive intact males used in this study were also given sexual performance tests to assess the relationship between partner preference and sexual performance. We predicted that there will be a positive relationship between preference for estrous females and sexual performance.

Materials and Methods

All animals were French Alpine goats group-housed by sex in an open barn with natural lighting and free access to an outdoor field. Sexually naive males had no contact with females after weaning. All other males and all females had prior sexual experience. Adults were greater than 2.5 yr of age and juveniles were 0.5 to 1.5 yr of age. Castrates were previously castrated (pre-puberty).

Experiment 1

Animals and treatments

Adult sexually experienced intact males ($n = 17$) and adult sexually naive castrates ($n = 11$) were tested for preference between groups of estrous and non-estrous females. The castrated males ($n = 9$) were retested for preference while receiving injections of testosterone propionate (25 mg; s.c.) three times a week two weeks prior to and during the partner preference tests (Steraloids Inc., Newport, RI). The intact males were administered sexual performance tests (see below).

Ovariectomized females ($n = 6$) and intact females ($n = 10$) were used as both the sexually receptive and non-receptive stimuli. Estrus was synchronized in intact females

with two injections of prostaglandin F2 α (10 mg, i.m.; Pfizer Inc., New York, NY) 11 days apart, with the second injection 49 h prior to the start of testing (Nuti et al., 1992). Estrus was induced in ovariectomized females with melengestrol acetate (0.5 mg, p.o.) 72 h and 48 h prior to estradiol injection (100 μ g, s.c.) (Steraloids Inc., Newport, RI).

Test apparatus

Small pens (2 m x 3 m), with a 1 m x 3 m incentive zone in front of each small pen, were located at opposite ends of the neutral zone (18 m x 1.5 m) (Fig. 10). The apparatus allowed for visual and olfactory investigation and limited physical contact between males and females. The small pens were partitioned into four equal smaller pens using wire paneling to prevent the females from interacting with one another, when needed. Two viewing towers, from which all data were recorded, were positioned behind each of the small pens. The apparatus was located outdoors in a field adjacent to where the subjects were housed.

Partner preference testing

Males were individually tested in a 5-min partner preference test. Time spent in each incentive zone and number of visits to each incentive zone were recorded. Video cameras were used to record female behavior.

Males were tested for preference between two groups of four females. The following treatment combinations were used: estrous females able to interact (E) versus non-estrous females able to interact (NE); estrous females unable to interact due to partitions (E_p) versus non-estrous females unable to interact (NE_p); and estrous females

able to interact (E) versus estrous females unable to interact (E_P). This was replicated using a switchback design to eliminate side bias, for a total of 6 trials. These trials were randomly conducted once a week over a 7 week period. During one week, the test had to be cancelled due to poor weather conditions. For the second part of the experiment there were fewer males being tested, so time permitted for the switchback to be tested on the same day, for a total of three test days. The trials were conducted in a random order over a 3-week period.

For each trial, half of the ovariectomized females and half of the intact females were randomly assigned to be in estrus; the remaining females were left untreated to serve as the non-estrous stimuli. E females were isolated from one another 14 h before testing to encourage mounting during the preference tests. Partner preference tests were conducted 14 h after ovariectomized females were treated with estradiol and 49 hours after intact females were treated with prostaglandin F2 α . The females' receptivity was observed to detect estrus 1 h before the start of testing using a sexually experienced male (not a subject male). The male was able to interact with the females but was on a lead to prevent him from mounting the females.

Sexual performance testing

Bucks were individually tested with a single estrous female in three 20-min sexual performance tests. Frequencies and latencies of sexual behaviors were recorded. Males were tested once a week for three weeks, and were tested in a random order on each test day. The tests were conducted in the males' home pen (approximately 4 m x 6 m), located in an enclosed barn. The males' home pen adjoined to an outdoor field, in which

males were held while the testing was conducted. Therefore, males could view the sexual performance tests as they were being conducted.

Experiment 2

Animals and treatments

In Experiment 1, the castrated males treated with testosterone served as a model for sexually naive intact males. At the time of that experiment we did not have a large enough group of sexually naive intact males to use for preference testing, however, two breeding seasons later we possessed a large enough group. For Experiment 2, juvenile sexually naive males ($n = 18$) were used. Ovariectomized females ($n = 20$) were used as both the sexually receptive and non-receptive stimuli. Estrus was induced as previously described, with half the females in estrus on each test day.

Partner preference and sexual performance testing

Males were tested for partner preference using the same test apparatus (Figure 10) and testing protocol used in Experiment 1. Video tape analysis could not accurately count female tail wagging, so it was not used for this experiment. Also, as we were unable to induce female-female mounting in Experiment 1, we eliminated the choices containing partitioned females. For this experiment, males were tested for preference between groups of four estrous and four non-estrous females. A switchback design was used to eliminate side bias, for a total of two trials which were conducted on the same day. All males were then administered six sexual performance tests (as described in Experiment 1) and the preference tests were repeated. The sexual performance tests were conducted

twice a week for 3 weeks. Including the preference tests, Experiment 2 was conducted over 5 consecutive weeks.

Statistical analysis

A preference score (PS) was calculated for each incentive zone: $PS = (\text{time in one incentive zone} / \text{time in both incentive zones}) \times 100$. Mean preference scores were compared with the Mann-Whitney U test ($P < 0.05$), and mean numbers of visits to each incentive zone were compared using a paired t -test ($P < 0.05$). For latencies, males that did not perform the behavior (i.e. first ejaculation) were assigned a score of 1200 sec (the length of the test). Correlations between measures of sexual performance and PS were analyzed using Spearman rank and Pearson product moment correlations. Measures of sexual performance were analyzed using repeated measures ANOVA. Variation around the mean preference scores is reported as the standard error of the proportion (\pm SEP). Statistical analysis was performed using NCSSTM software (NCSS Statistical Software, Kaysville, UT).

Results

Experiment 1

Sexually experienced intact males preferred E versus NE females ($P < 0.001$; Figure 11A) and made more visits to the E female incentive zone ($P < 0.01$; Figure 12A). They also preferred E_P versus NE_P females ($P < 0.001$; Figure 11B) and showed no preference for E versus E_P females ($P > 0.05$; Figure 11C). Sexually naive castrates

showed no preferences for any of the treatment groups ($P > 0.05$; Figure 11A, B, C). Sexually naive castrates treated with testosterone preferred E versus NE females ($P < 0.001$; Figure 11A). They also preferred E_p versus NE_p females (Figure 11B) but showed no preference for E versus E_p females ($P > 0.05$; Figure 11C). They also made significantly more visits to the E_p female incentive zone for E_p versus NE_p females ($P < 0.01$; Figure 12B). There were no correlations between measures of sexual performance and PS.

Experiment 2

Males preferred E versus NE females ($P < 0.05$; Figure 13) and made more visits to the E female incentive zone ($P < 0.05$; Figure 14) before and after sexual experience. Male sexual performance remained relatively constant throughout the six sexual performance tests (Figure 15), and there were no correlations between sexual performance and PS.

Discussion

It appears that a group of estrous females is sufficient to elicit a preference in sexually experienced, intact males, as they preferred groups of estrous females over non-estrous females, regardless of whether the estrous females could interact with one another. Furthermore, they showed no preference between two groups of estrous females in which one of the groups could not interact with one another. As hypothesized, sexually naive castrates showed no preferences. The preferences exhibited by the sexually naive,

TP-treated castrates are the same as the preferences exhibited by the sexually experienced, intact males. This suggests that TP alone is able to elicit a preference for estrous females and that male goats exhibit a preference for estrous females before ever mating. This is further supported by the results of Experiment 2, in which sexually naive, intact males preferred estrous females both before and after sexual experience.

In Chapter 2, sexually experienced males did not show a distinct preference for estrous state (E vs. NE) when single females were used. We feel this difference in preference exhibited by males in these two studies is due to the use of groups of females in the current study. We do not feel that the lack of preference in Chapter 2 was due to the sample size of 13 males, as the current study demonstrated significant preferences in three groups of males with sample sizes of 9, 17, and 18.

The most frequent observable behavior exhibited by the groups of estrous females was tail wagging. Despite efforts to encourage female-female mounting, only a few incidences of this behavior occurred. Tail wagging among the group was almost constant; one or more females were tail wagging at any given time during the preference test. The level of tail wagging was considerably higher than the previous experiments in which only one female was used (Appendices A-C) and was similar to the level of tail wagging exhibited by flutamide-treated estrous females (Chapters 2-3). This assessment of tail wagging is from observation only, as analysis of the video taped tests could not accurately count tail wags. The group dynamic of the females appears to encourage tail wagging. Isolated females tend to tail wag less in the presence of a male than a group of females in the presence of a male (personal observation). There are also individual variations among females regarding frequency of tail wagging. A group of females may

appear to tail wag more because there are more females likely to tail wag. Furthermore, tail wagging in a group may serve to attract the male, as opposed to a solitary female that has the full attention of the male. These findings further support the idea that tail wagging is an important attractive and proceptive behavior affecting partner preference and sexual motivation in sexually naive and sexually experienced male goats.

In Experiment 2, the sexual performance of the juvenile, sexually naive males remained relatively constant throughout the six sexual performance tests. Male sheep require sexual experience as juveniles, as males raised in all-male groups exhibit sexual impotence and low ejaculatory rates as adults (Price et al., 1994; Stellflug and Berardinelli, 2002). However, it has been demonstrated that juvenile cattle (Price and Wallach, 1990), swine (Hemsworth et al., 1977), and goats (Price et al., 1998) do not require sexual experience to attain full sexual performance as adults. In contrast, Imwalle and Katz (2004a) reported that juvenile, sexually naive goats display separation anxiety when removed from pen mates and can require up to six sexual interactions with receptive females before attaining consistent sexual performance scores. They attributed this difference to their use of unrestrained females, as Price et al. (1998) used restrained females for the sexual performance tests and both studies tested males outside of their home pens. The current study was specifically designed to test males in their home pen in order to avoid any effects of separation anxiety on sexual performance. Furthermore, unrestrained females were used. The sexual performance of the juvenile, sexually naive males remained relatively constant throughout the current study, indicating that conducting the test in the home pen alleviated any effects separation anxiety may have on sexual performance. From an animal production standpoint, if breeders do not wish to

measure sexual performance repeatedly, the tests should be conducted in the males' home pen.

There were no significant correlations between measures of sexual performance and partner preference for sexually experienced or sexually naive males, indicating that partner preference is not a reliable indicator of sexual performance in male goats. Goats exhibit promiscuous mating behavior, in which no exclusive mating rights exist between individuals. Promiscuity selects for high sexual performance in males, with most males exhibiting near maximal performance (Katz, 2008). However, "relaxation of natural selection" can occur in captive populations in which males with poor sexual performance are allowed to reproduce, such as in single-sire breeding programs (Price, 1984). It appears that this has not occurred within our population of goats, as there is little variation in sexual performance among our males. However, the way in which sexual performance was assessed was only over a 20 minute time period, which is common practice in the animal production industry. If most males are performing at near maximal performance for this limited time period, it would be difficult to correlate sexual performance with partner preference in a small population of males. Perhaps a more thorough assessment of sexual performance could be correlated to partner preference.

We conclude that preference for estrous females is dependent on hormonal state, not sexual experience, in male goats. Also, partner preference is not a reliable indicator of sexual performance in male goats. This experiment further supports the hypothesis that female tail wagging is an important attractive and proceptive behavior affecting partner preference in sexually naive and experienced male goats.

Figure 10

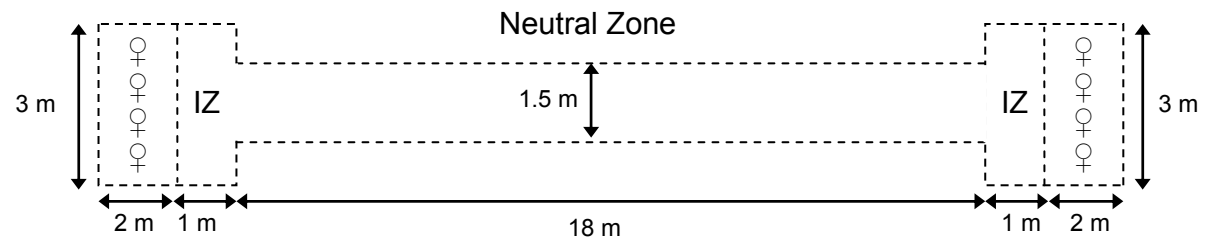


Figure 10. Partner preference testing apparatus. Dashed lines indicate wire fencing. IZ = Incentive Zone.

Figure 11

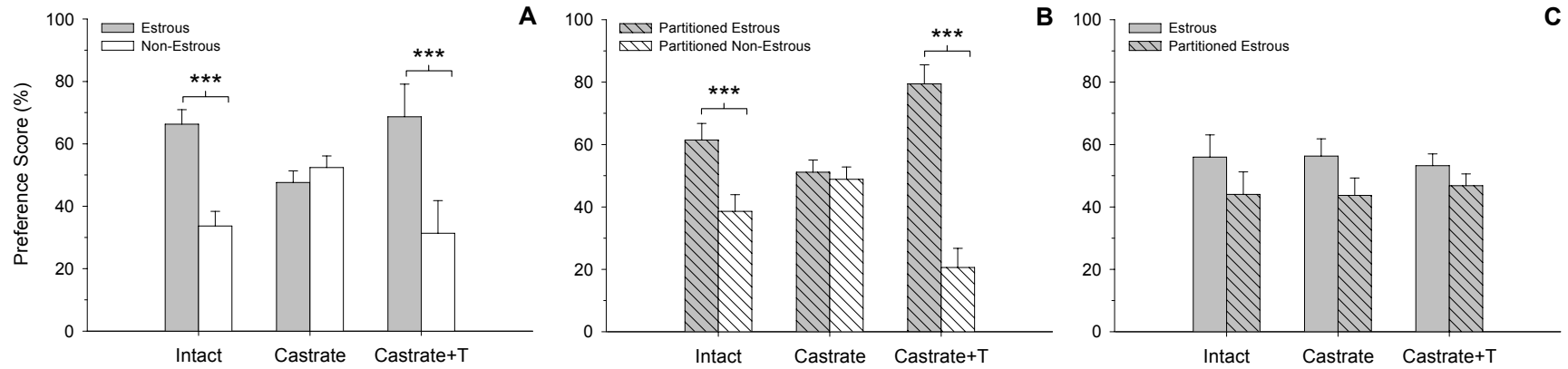


Figure 11. Mean (\pm SEP) preference scores by female treatment pairs for sexually experienced intact males and sexually naive castrates, with or without testosterone propionate treatment. Females that were in partitioned pens could not interact with one another. Bars with superscripts differ (Mann-Whitney U test, *** = $P < 0.001$).

Figure 12

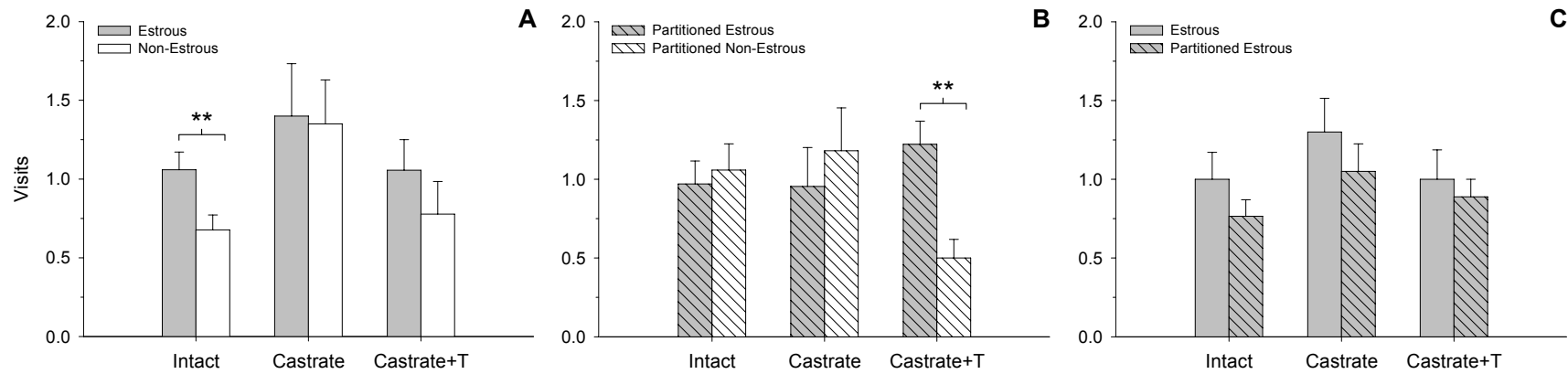


Figure 12. Mean (\pm SEM) visits by female treatment pairs for sexually experienced intact males and sexually naive castrates, with or without testosterone propionate treatment. Females that were in partitioned pens could not interact with one another. Bars with superscripts differ (paired t-test, $** = P < 0.01$).

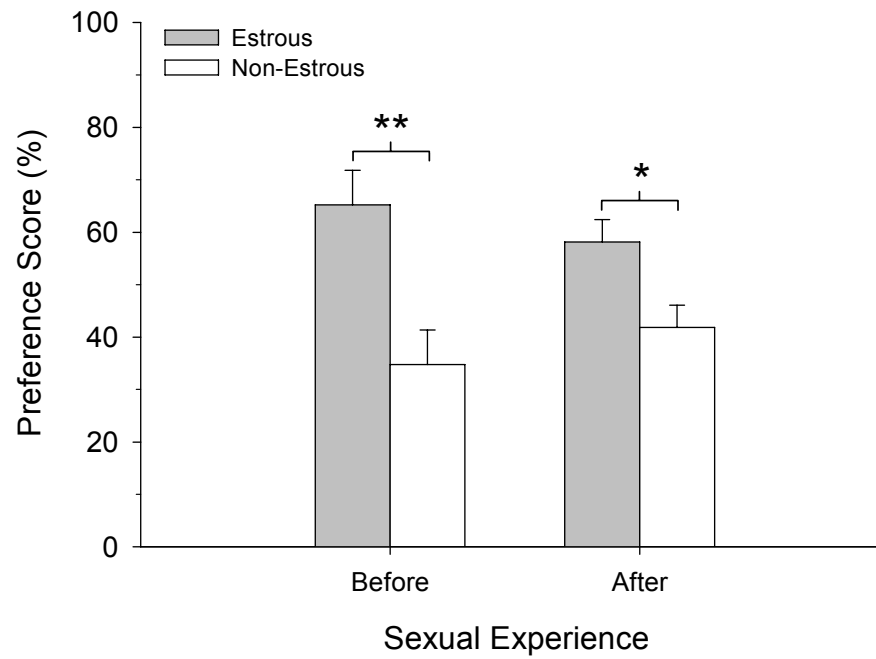
Figure 13

Figure 13. Mean (\pm SEP) preference scores by female treatment pairs for sexually naive males, before and after sexual experience. Bars with superscripts differ (Mann-Whitney U test, ** = $P < 0.01$; * = $P < 0.05$).

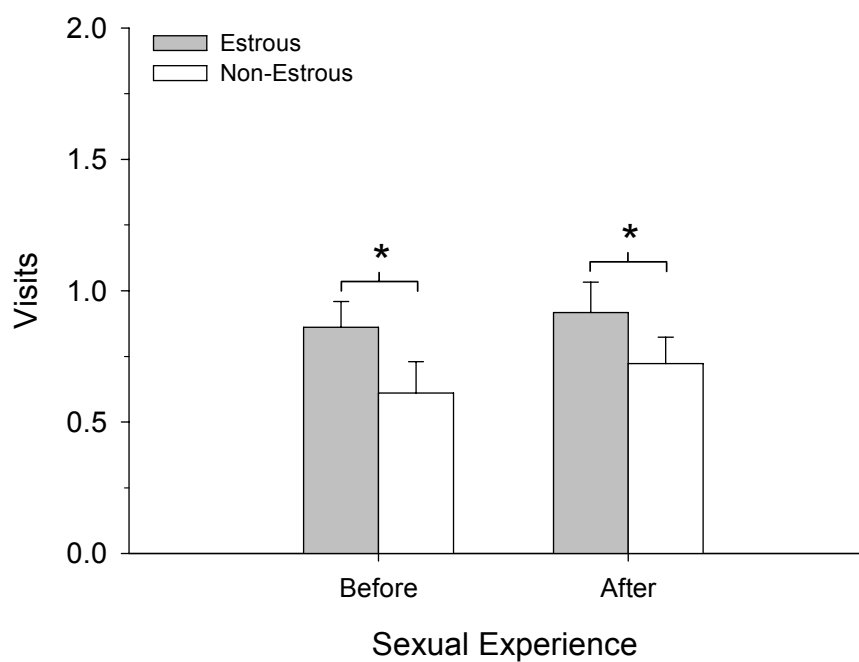
Figure 14

Figure 14. Mean (\pm SEM) visits by female treatment pairs for sexually naive males, before and after sexual experience. Bars with superscripts differ (paired t-test, * = $P < 0.05$).

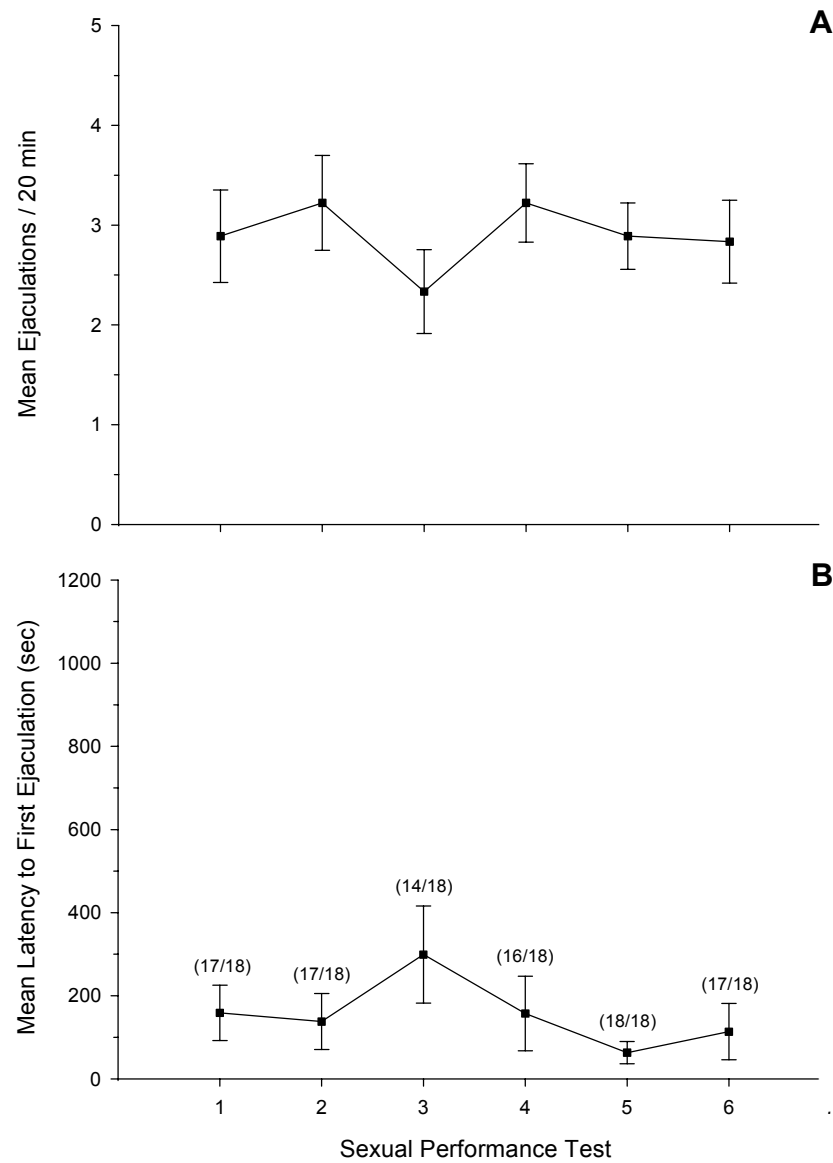
Figure 15

Figure 15. Mean (\pm SEM) frequency of ejaculation (A) and mean latency to first ejaculation (B) for sexually naive males during six sexual performance tests, each lasting 20 min. Ratios in parentheses are the proportion of males ejaculating.

CHAPTER 5

Summary and Conclusions

These studies have demonstrated that the tail wagging behavior of female goats in estrus plays an important role in the sexual motivation, as measured by partner preference, and sexual performance of male goats. The objectives of these studies were (1) to determine if males prefer the tail wagging behavior of estrous females; (2) to determine if the tail wagging behavior of estrous females can stimulate sexual performance in males; (3) to determine if male preference for the tail wagging behavior of estrous females is dependent on hormonal state or sexual experience of the male; and (4) to determine if strength of preference for estrous females can predict sexual performance in sexually experienced and sexually naive males.

It was hypothesized that males would prefer females that tail wagged more frequently than females that tail wagged less frequently. Sexual motivation is stimulated by the presence of a suitable mate (Beach, 1956). Approach behaviors by the male are activated by appropriate incentives by the female, therefore sexual motivation is an incentive motivation. These studies demonstrate that tail wagging is an important incentive property of female goats in estrus, as it is able to elicit partner preference in sexually naive and experienced males. By Beach's (1976) definitions, tail wagging is both an attractive and proceptive behavior in female goats. When tail wagging initiates approach behaviors from a male it can be thought of as attractivity, and when the female continues to display the behavior to maintain interactions with the male it can be thought of as proceptivity.

It was hypothesized that preference for estrous females is dependent on hormonal state and not sexual experience. This hypothesis was supported by the findings of this thesis, as testosterone propionate treatment was able to elicit a preference in sexually naive, castrated males which previously showed no preference among females. In various species, different measures of sexual motivation for sexually receptive females can be reduced or eliminated following castration, indicating that gonadal steroid actions in the brain are necessary for the development and/or maintenance of these behaviors (Pfaus et al., 2003). For example, sexually naive and experienced male rats prefer sexually receptive versus non-receptive females, and castration abolishes these preferences while testosterone replacement restores them (Meyerson and Lindstrom, 1973; Hetta and Meyerson, 1978; Merkx, 1984; Edwards and Einhorn, 1986; Agmo, 2003).

It is important to note that males showed a preference for tail wagging and not estrous state. It appears that this preference is due to the visual cue of tail wagging; however, it remains unclear if tail wagging also causes olfactory stimulation in males from the wafting of pheromones. Shank (1972) believes that tail wagging functions to fan vaginal odors in the direction of the male in order to relay a female's estrous state or to entice the male to pursue the female. However, males showed no preference between estrous and non-estrous females whose tail wagging behavior was not different. It is presumed that the main difference between these females is olfactory cues. Therefore, visual cues may be more important to a male's ability to identify estrous females than olfactory cues.

Similar findings were reported in cattle, in which sexually experienced males showed no preference between an estrous and non-estrous female (Geary et al., 1991).

However, males preferred a pair of females engaging in female-female mounting, a visual cue of estrus, over non-estrous females and estrous females unable to mount one another (Geary and Reeves, 1992). Cattle and goats share a similar social structure, in which females form groups in the center of a home range and adult males live on the periphery (Crook, 1969; Geist, 1964; Kilgour et al., 1977). Female goats and cattle in estrus form sexually active groups that engage in female-female mounting, as well as other behaviors (Blockey, 1978; Kilgour et al., 1977; Shank, 1972). Visual stimuli may be more important in locating estrous females as olfactory cues may not travel great distances across a home range. In contrast, rodents display preferences for the odors of sexually receptive versus non-receptive females (Carr et al., 1970; Stern, 1970; Bakker et al., 1996). This difference is most likely due to the environment in which each species lives. Most rodents are nocturnal and live in dark, enclosed spaces. Therefore, they would need to rely on olfaction to find a mate.

These studies demonstrate that male goats exhibit enhanced sexual performance after viewing estrous females that display high rates of tail wagging, supporting our hypothesis. Moreover, this finding further supports the argument that tail wagging is a visual cue. The sexual performance of male goats is also enhanced when males view females mounting one another (Shearer and Katz, 2006). There were no effects on sexual performance when males viewed estrous females with low rates of tail wagging in either of these studies. Male goats and cattle show enhanced sexual performance when they are allowed to watch conspecifics mating (Mader and Price, 1984; Price et al., 1984; Shearer and Katz, 2006). These effects may have resulted from selective pressures to identify estrous females from a distance. Furthermore, there are adaptive advantages of these

effects. Males that can identify and be aroused by estrous females would be able to locate and mate with these females sooner and more frequently than less responsive males. These males may also sire more offspring, because the receptivity of female goats and cattle is reduced after repeated matings (Price et al., 1998).

Tail wagging may serve other functions in addition to being a visual cue to attract males from a distance, as female goats continue to tail wag in the presence of a male. Female goats will repeatedly approach a male, tail wag, move away and then re-approach the male. They will repeat this sequence until the male pursues or performs a sexual behavior. Beach (1976) stated that this behavior sequence may function to correctly orient the male in a posterior position to allow him to correctly mount the female. The tail wagging behavior of female goats is unique to the approach-withdrawal pattern. It may act to arouse the male or waft pheromones, as proposed by Shank (1972). Flehmen aids in the transport of fluid-borne substances, such as pheromones, to the vomeronasal organ so males can determine the estrous state of the female (Gelez and Fabre-Nys, 2004). Male goats display flehmen in response to the olfactory investigation of a female's expelled urine or perineal region (Shank, 1972). A female may continue to tail wag as the male investigates her perineal region. Under such close contact, it is unlikely that tail wagging would further aid in the transmission of pheromones to the male through a wafting action. Females also continue to wag their tails as a male mounts, which may help to stimulate and guide the penis for intromission.

Tail wagging may also play a role in a female's reproductive fitness. There are individual variations in the amount of tail wagging that estrous females display. Females that tail wag more may have a better chance of being impregnated since tail wagging is

able to attract and arouse males. Furthermore, isolated females tend to tail wag less in the presence of a male than a group of females in the presence of a male. When females need to compete for the attention of males, selection may favor the evolution of signals that indicate their fecundity and/or attract the attention of males (Clutton-Brock, 2009). Tail wagging while in a group may serve to attract the male, as opposed to a solitary female that has the full attention of the male.

It has been reported that juvenile males (Price et al., 1998) do not require sexual experience to attain full sexual performance as adults. In contrast, Imwalle and Katz (2004a) reported that juvenile males display separation anxiety when removed from pen mates and can require up to six sexual interactions with receptive females before attaining consistent sexual performance scores. This difference was attributed to their use of unrestrained females, as Price et al. (1998) used restrained females. Both studies tested males outside of their home pens. In the current study, the sexual performance of the juvenile, sexually naive males remained relatively constant throughout the six sexual performance tests conducted in the males' home pen with an unrestrained female. This indicates that conducting the test in the home pen alleviated any effects separation anxiety may have on sexual performance.

These studies do not support our hypothesis that strength of partner preference can predict sexual performance in males. Partner preference was not a reliable indicator of sexual performance in sexually experienced or naive male goats, most likely due to little variation in sexual performance among our small population of males. The promiscuity of goats naturally selects for high sexual performance in males, with most males exhibiting near maximal performance (Katz, 2008). "Relaxation of natural

selection” can occur in captive populations because males with poor sexual performance are allowed to reproduce (Price, 1984); however, it appears that this has not occurred within our population of goats.

In conclusion, tail wagging is an important attractive and proceptive behavior affecting partner preference and sexual performance in the male goat. Preference for estrous females is dependent on hormonal state and not sexual experience. Lastly, partner preference is not a reliable indicator of sexual performance in male goats.

APPENDIX A

Preliminary Experiment 1:

Failure of the straight-arm runway to assess sexual motivation in male goats

This experiment used a straight-arm runway to assess sexual motivation in sexually naive and experienced male goats. An estrous female or non-estrous female was used as the choice stimuli. It was hypothesized that if sexual motivation develops early in the male goat, sexually naive males would have faster run times (i.e. more motivation) for estrous females, and slower run times (i.e. less motivation) for non-estrous females. It was also hypothesized that sexually experienced males would have faster run times for estrous females, and slower run times for non-estrous females.

Methods

All animals were French Alpine goats group-housed by sex in an open barn with natural lighting and free access to an outdoor field. Sexually naive males had no contact with females after weaning. All other males and all females had prior sexual experience.

Sexually naive males ($n = 6$) 8 months of age and sexually experienced males ($n = 10$) 2 to 3 years of age were used. Intact females ($n = 5$) were used as the sexually receptive stimuli. Estrus was induced with two IM injections of prostaglandin F2 α (10 mg/goat) 11 days apart, with the second injection 51 h prior to the start of testing (Nuti et al., 1992). Ovariectomized females ($n = 2$) were left untreated and used as the sexually non-receptive stimuli. Estrous detection was conducted 1 h before the start of testing

using a sexually experienced male (not a subject male) on a lead to prevent mounting.

The female's behavior, such as tail wagging and standing to be mounted, was observed to determine if she was in estrus.

The straight-arm runway was made of plywood, consisting of a completely closed start box (1.3 m x 1 m x 1 m), a runway (24 m x 1.6 m x 1.3 m) and a goal box (2.6 m x 1.6 m x 1.3 m) (Figure 1). Solid, wooden doors were located at both ends of the start box and at both ends of the goal box. A wire fence divided the goal box into two equal halves, with the end half occupied by the stimulus. The male had to run a total of 25.3 m until it reached the stimulus in the goal box. A viewing tower, from which all data was recorded, was positioned at the end of the goal box. The apparatus was located outdoors in a field adjacent to where the subjects were housed.

Figure 1: Experiment 1 Testing Apparatus

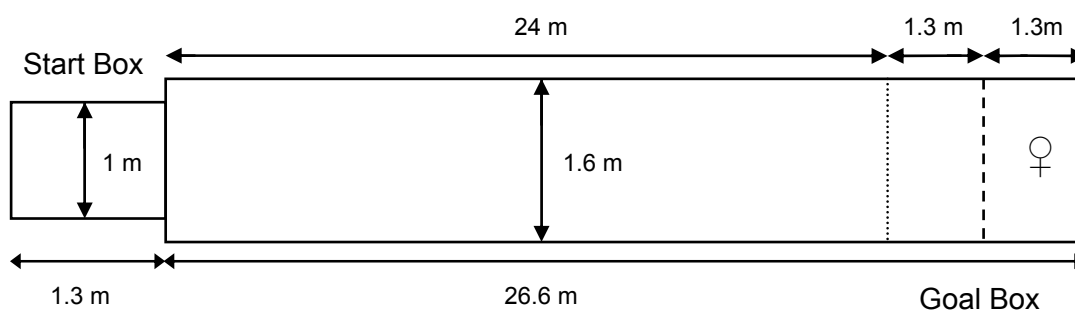


Figure 1. Dotted line indicates solid wood door that could be open or closed and dashed line indicates wire fence.

All subjects were acclimated to the testing apparatus prior to the start of the experiment. Subjects were first exposed to the apparatus in groups. The size of the group was reduced until the subject was alone. A subject was considered acclimated to the apparatus once it could remain in the apparatus by itself with no apparent signs of distress.

The motivation test consisted of two parts. First, the male was placed in the goal box for 5 min during which he could see, hear and chemo-investigate the female, if present. A wire fence prevented mounting. Next, the male was placed in the start box and released into the runway. The time required for the male to reach the goal box was recorded. Stops and retreats were also recorded. A retreat was defined by the male moving toward the start box rather than the goal box. A time limit of 5 min was set; therefore if the male did not reach the goal box within the time limit, he was removed from the runway and assigned a score of 5 min.

Five treatments were used. Each male was tested once for each treatment for a total of 5 trials over 5 weeks. The treatments used were an empty goal box, an estrous female or a non-estrous female. For these treatments the stimulus remained the same for both parts of the test, i.e the male could investigate the goal before being run through the test. Treatments were also used in which the stimulus was removed from the goal box before the male was released into the runway. These treatments were an estrous female followed by an empty goal box or non-estrous female followed by an empty goal box.

Mean run times and mean stops and retreats were compared using single factor ANOVA. Comparisons were also made based on sexual experience using single factor

ANOVA. Post-hoc differences in run time and stops and retreats were compared using Duncan's Multiple Comparison Tests ($P < 0.05$).

Results

An effect of goal box stimulus for time to reach the goal box was seen for all males ($F_{4,80} = 3.44$; $P < 0.05$) (Figure 2A). Males exposed to the empty goal box took significantly longer to reach the goal box ($P < 0.05$). The goal box stimulus of a non-estrous female followed by an empty goal box was not significantly different from any of the other stimuli used. An effect of goal box stimulus for the number of stops and retreats before reaching the goal box was seen for all males ($F_{4,80} = 3.44$; $P < 0.01$) (Figure 2B). Males exposed to the empty goal box had significantly more stops and retreats ($P < 0.05$).

There was no significant effect of sexual experience on run time ($P > 0.05$) (Figure 2C) but there was a tendency for sexual experience to affect the number of stops and retreats ($F_{1,80} = 3.69$; $P < 0.06$) (Figure 2D). With most goal box stimuli, sexually naive males made more stops and retreats than sexually experienced males.

Figure 2. Mean (\pm SEM) run times and stops and retreats for all stimuli

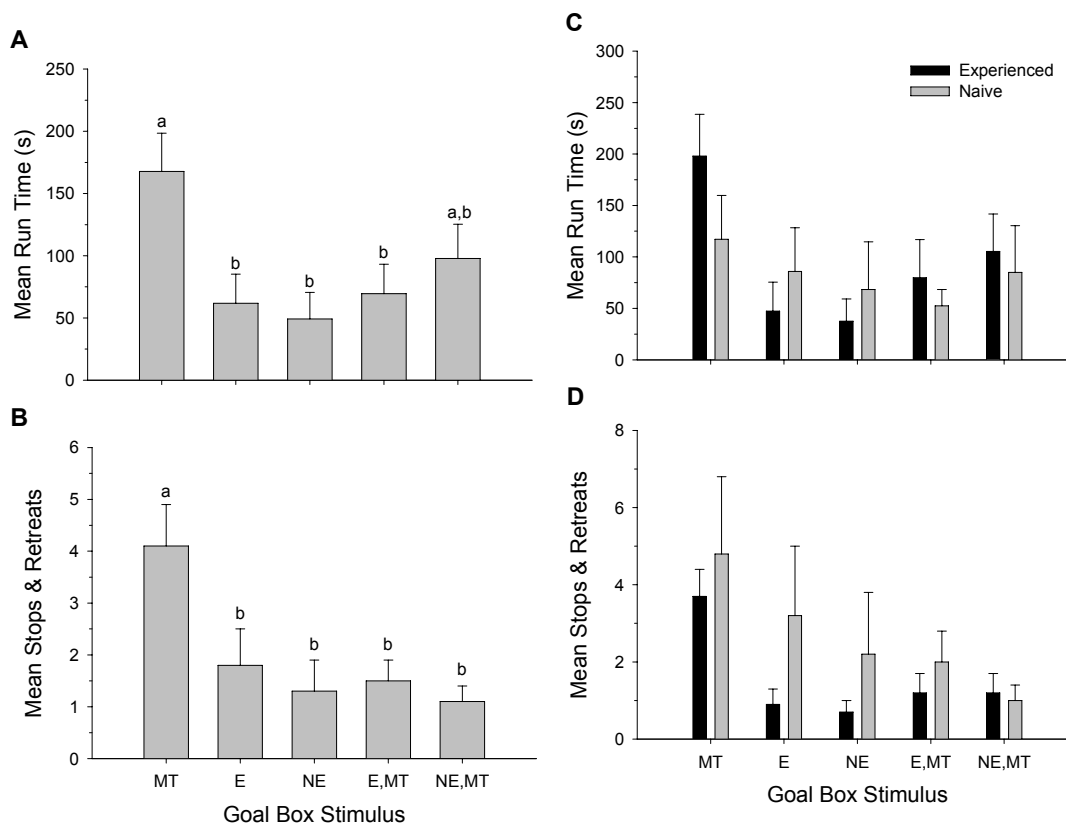


Figure 1. Mean (\pm SEM) run times and stops and retreats for all stimuli collapsed across sexual experience (A,B) and by sexual experience (C,D). Bars with different superscripts differ (Duncan's, $P < 0.05$). MT – empty goal box; E – estrous female; NE – non-estrous female; E,MT – estrous female followed by and empty goal box; NE,MT – non-estrous female followed by and empty goal box.

Discussion

The results do not support the hypothesis that males would be more motivated to run to an estrous female than a non-estrous female. However, male goats are more motivated to run to another goat than an empty goal box. There was no effect of sexual

experience on run time, but sexually experienced males did make fewer stops and retreats, possibly suggesting that copulation may aid in reinforcing motivation. This effect could also be due to age differences between the sexually naive and experienced goats, with the naive males being one to two years younger. Goats are herd animals and appear to be motivated to spend time with another goat, regardless of the animal's estrous state. The experiment was designed to try to eliminate this effect by exposing the male to a female first and then letting it run to an empty goal box. Males were slightly less motivated to run to the empty goal box after interacting with a non-estrous female, suggesting that a difference in motivation may exist. It appears that a straight-arm runway is not adequate to test sexual motivation in goats due to the social nature of goats. However this design successfully reveals preferences in non-herd animals, such as rats, in which sexually naive and experienced male rats run significantly faster for an estrous female than a non-estrous female (Lopez et al., 1999).

APPENDIX B

Preliminary Experiment 2:

Use of a Y-maze to assess sexual motivation in male goats

This experiment, using a Y-maze, was designed to eliminate the social aspect of motivation, because the males would have to make a choice between an estrous and non-estrous female rather than just being motivated to be with another goat. A Y-maze assesses partner preference as an indirect measure of sexual motivation. Time spent in proximity to a stimulus (stimulus proximity) or the total number of times the subject chooses a stimulus (discrete trials) is a measure of preference for that stimulus. It was hypothesized that males would prefer to spend time with, and would make more choices to spend time with an estrous female versus a non-estrous female, irrespective of sexual experience. The same experiment was then repeated after all males were given sexual performance tests. It was hypothesized that sexual performance testing would either elicit or strengthen a preference for an estrous female.

Methods

Sexually naive males ($n = 4$) 1.5 years of age and sexually experienced males ($n = 8$) 2.5 to 3.5 years of age were used. Intact females ($n = 5$) were used as the sexually receptive stimuli. Estrus was induced with two IM injections of prostaglandin F_{2α} (10 mg/goat) 11 days apart, with the second injection 51 h prior to the start of testing (Nuti et al., 1992). Ovariectomized females ($n = 2$) were left untreated and used as the sexually

non-receptive stimuli. Estrous detection was conducted 1 h before the start of testing using a sexually experienced male (not a subject male) on a lead to prevent mounting. The female's behavior, such as tail wagging and standing to be mounted, was observed to determine if she was in estrus.

A Y-maze was constructed of plywood, consisting of a completely closed start box (1.3 m x 1 m x 1 m) and 2 goal boxes (1.2 m x 1.5 m x 1.2 m) (Figure 1). The arms measured 10 m x 1.5 m x 1.2 m. The start box was placed 2.4 m behind the branch point of the Y. Solid, wooden doors were located at the beginning of each arm of the Y. These doors could remain open allowing access to the arms or could be closed blocking access to the arms. Wire fences partitioned the goal boxes from the arms of the Y. A viewing tower, from which all data was recorded, was positioned between the two goal boxes. The apparatus was located outdoors in a field adjacent to where the subjects were housed. All subjects were acclimated to testing apparatus as previously described.

Males were individually tested for partner preference between an estrous female and a non-estrous female located in goal boxes at each end of the Y using stimulus proximity and discrete trials testing paradigms (Kelliher and Baum, 2002). For the stimulus proximity tests, the male was released from the start box and given 30 min to explore the Y-maze. The amount of time spent in proximity to each goal box was recorded. As the males did not explore both arms of the maze the SP tests were discontinued. For the discrete trials, the male was released from the start box and given 2 min to approach either of the two goal boxes (free trial). The choice was recorded and the male was placed back in the start box. The door to the arm just chosen was closed blocking access to that arm. The male was then released and given 2 min to approach the

other goal box (guided trial). A discrete trial consisted of alternating six free and five guided trials. This was replicated 4 times with 2 trials per week for a total of 2 weeks. A switchback design was used to eliminate side bias. Only data from the 20 free trials which followed guided trials were analyzed.

Figure 1: Experiment 2 Testing Apparatus

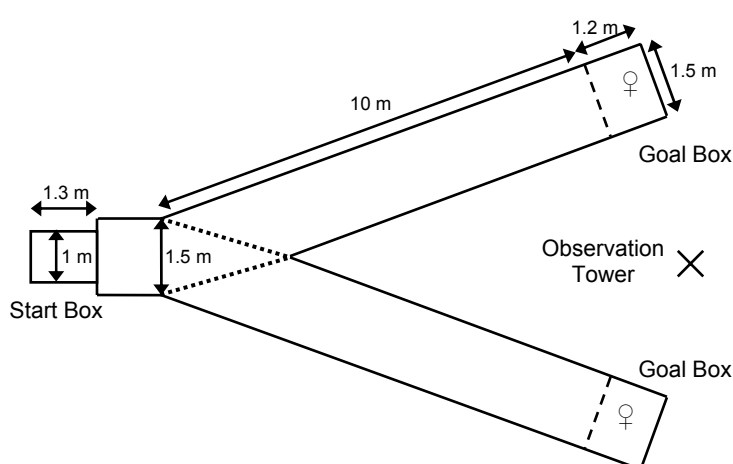


Figure 1. Dotted lines indicate solid wood doors that could be open or closed and dashed lines indicate wire fences.

Following the preference tests all males were given sexual performance tests with a receptive female. The intact females from the preference tests were used. The tests were conducted in a pen (10 m x 4 m) located in an enclosed barn. During each test, a male was placed into the test pen with an unrestrained, receptive female for 15 min. Latency to first mount and all ejaculations was recorded, as well as the total number of mounts and ejaculations. This was repeated once a week for 5 weeks, for a total of 5 sexual

performance tests. However, one week the tests had to be discontinued due to a lack of females in estrus, resulting in only 5 of the 12 males receiving a sexual performance test. Subsequent to the sexual performance tests, the discrete trials testing paradigm was repeated.

Statistical analysis was performed using NCSS™ software (NCSS Statistical Software, Kaysville, UT). Mean choices for estrous or non-estrous females were compared between sexual experience groups using a paired t-test ($P < 0.05$). Spearman's Rank Correlation was used to determine if there were correlations between preference scores and data collected during sexual performance tests.

Results

The stimulus proximity tests were discontinued because the males did not explore both arms of the Y-maze. For the discrete trials, sexually naive males preferred an estrous female (86% of choices; $P < 0.01$), whereas experienced males showed no preference between an estrous female and a non-estrous female (57% of choices; $P > 0.05$) (Figure 2A). After receiving sexual performance tests, experienced males preferred an estrous female (71% of choices; $P < 0.05$), whereas the previously sexually naive males showed only a tendency to prefer an estrous female (65% of choices; $P < 0.06$) (Figure 2B). Rank of mounting frequency was positively correlated with rank of choices for an estrous female for sexually naive males in the discrete trials that preceded the sexual performance tests ($R_s = 1.0$, $P < 0.05$). For all males after sexual performance testing, there was a tendency for a positive correlation between rank of choices for an estrous female and rank of ejaculations ($R_s = 0.49$, $0.05 > P > 0.1$).

Figure 2. Mean (\pm SEM) choices for an estrous or non-estrous female by sexual experience

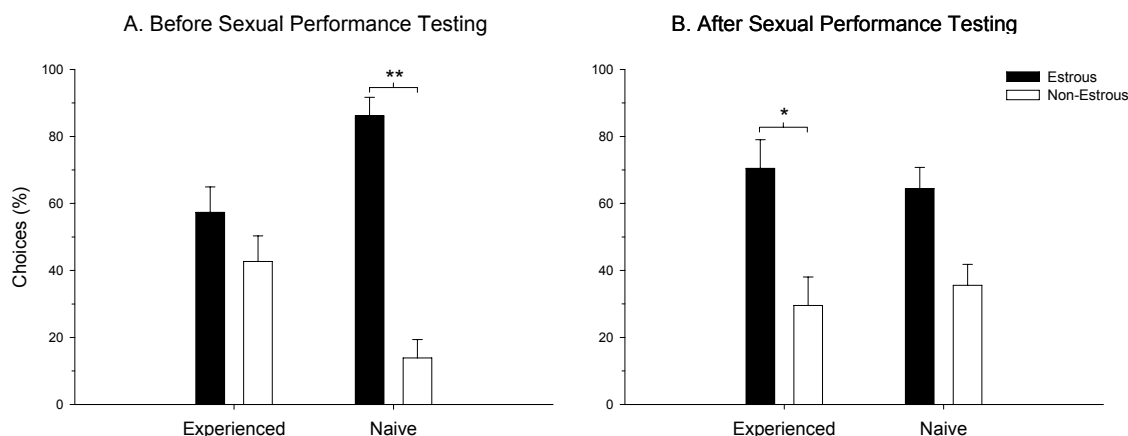


Figure 2. Mean (\pm SEM) choices for an estrous or non-estrous female by sexual experience before (A) and after (B) sexual performance testing. Bars with superscripts differ (paired t-test, * = $P < 0.05$, ** = $P < 0.01$).

Discussion

The stimulus proximity tests were discontinued because the males did not explore both arms of the Y-maze and were therefore not making a choice between the two females. Our lab used the same stimulus proximity tests to assess partner preference in female goats, with estrous females preferring to spend time with a male over an estrous or diestrous female, and diestrous females displaying no preference for the three stimuli (Margiasso and Katz, 2005).

The discrete trials ensured that a choice was made after at least one exposure to each stimulus. For the discrete trials, sexually naive males preferred an estrous female, whereas experienced males showed no preference. However, after all males received

sexual performance tests the experienced males preferred an estrous female, whereas the previously sexually naive males showed only a tendency to prefer an estrous female. This lack of significance for the previously sexually naive males could be due to the small sample size of only four males. Furthermore, the results may have been confounded by age, as the naive goats were one to two years younger than the experienced males. It appears that sexual activity was able to elicit a preference in the sexually experienced males, but was not able to maintain a preference in the sexually naive males. Rank of mounting frequency was positively correlated with rank of choices for an estrous female for sexually naive males in the discrete trials that preceded the sexual performance tests. This suggests that strength of preference for an estrous female can predict mount frequency for sexually naive males. However, it should be noted that only four males were tested. Overall, the discrete trials testing paradigm shows promise as a sexual motivation assessment tool as it ensures that a choice is made after at least one exposure to each stimulus. However, from a practical standpoint the repetitive nature of the test makes it tedious and time consuming to conduct.

APPENDIX C

Preliminary Experiment 3: Use of a partner preference test to assess sexual motivation in male goats

This experiment utilized a test pen to assess sexual motivation using stimulus proximity as a measure of partner preference. Unlike the Y-maze, this experiment was designed so that the male could view both female stimuli at the same time, with a defined incentive zone (timed zone) in front of each pen containing a female and a neutral zone (untimed zone) located between incentive zones. It was hypothesized that males would prefer to spend more time with an estrous female than a non-estrous female. For the last trial of this experiment a sexual performance test immediately preceded the preference test. It was hypothesized that sexual performance testing would either elicit or strengthen a preference for an estrous female.

Methods

Sexually experienced males ($n = 7$) 2.5 to 4.5 years of age were used. Ovariectomized females ($n = 6$) were used as both the sexually receptive and non-receptive stimuli. Estrus was induced with sc injections of progesterone 72 h (10 mg/goat) and 48 h (5 mg/goat) prior to estradiol (100 µg/goat) injections (Billings and Katz, 1997). For each test, half the females were randomly chosen to be left untreated and used as the sexually non-receptive stimuli. Testing began 14 h after females were treated with estradiol. Estrous detection was conducted 1 h before the start of testing using a sexually experienced male (not a subject male) on a lead to prevent mounting. The

female's behavior, such as tail wagging and standing to be mounted, was observed to determine if she was in estrus.

Small pens (2 m x 3 m) were located at opposite ends of a test arena (10 m x 4 m) located in an enclosed barn (Figure 1). A 1.2 m incentive zone was defined in front of each small pen, with a 4 m neutral zone located between incentive zones.

Figure 1: Experiment 3 Testing Apparatus

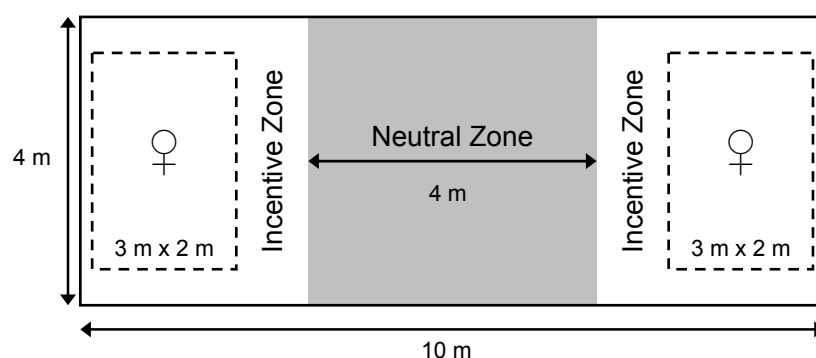


Figure 5. Dashed lines indicate wire fence pens.

Males were individually tested in 10 min tests for partner preference between an estrous female and a non-estrous female located in the small pens. Time spent in each incentive zone and number of entries to each incentive zone was recorded. This was repeated once a week for 4 weeks, for a total of 4 trials. A switchback design was used to eliminate side bias. A fifth trial was conducted in which a 10 min preference test was preceded by a 5 min sexual performance test. During the sexual performance test, males could freely interact with the estrous and non-estrous females that would be used in the

preference test. Latency to first mount and all ejaculations was recorded, as well as the total number of mounts and ejaculations.

Statistical analysis was performed using NCSSTM software (NCSS Statistical Software, Kaysville, UT). A preference score was calculated for each incentive zone: time spent in incentive zone/(time spent in the estrous female incentive zone + time spent in the non-estrous female incentive zone). Mean preference scores were compared with the Mann-Whitney U test ($P < 0.05$). Mean number of entries to each incentive zone were compared using a paired t-test ($P < 0.05$). Spearman's Rank Correlation was used to determine if there were correlations between preference and data collected during sexual performance tests.

Results

Males tended to prefer an estrous female over a non-estrous female for trials 1-4 ($P = 0.055$) (Figure 2). For trial 5, immediately after a sexual performance test, males showed no preference for an estrous versus non-estrous female ($P > 0.05$) and rank of mounting frequency was negatively correlated with rank of preference for an estrous female ($R_s = -0.72$, $P < 0.05$).

Figure 2. Mean (\pm SEM) preference scores for an estrous or non-estrous female

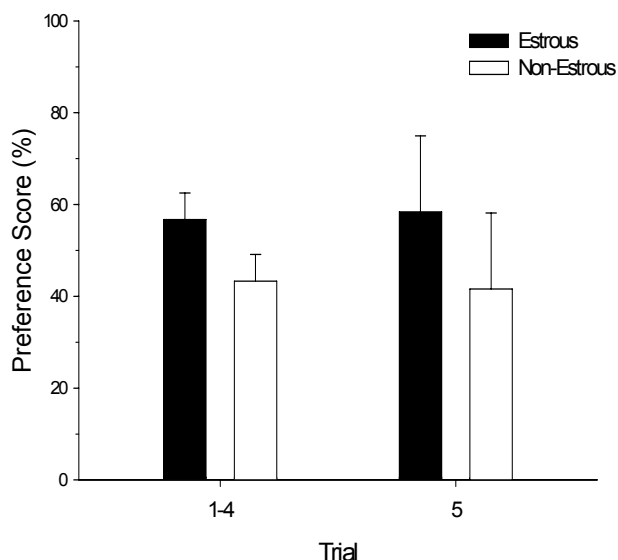


Figure 2. Mean (\pm SEP) preference scores for an estrous or non-estrous female for trials 1-4 and trial 5 (immediately after a sexual performance test).

Discussion

Males showed no significant preference for an estrous or non-estrous female for all trials, including a trial after a sexual performance test. For trials not involving a sexual performance test there was a tendency for males to prefer an estrous female over a non-estrous female, which may indicate that a preference exists. A lack of preference may be due to the close proximity of the females (6.4 m apart). Males were observed standing in the neutral zone looking back and forth between the two females. Similar results were obtained with bulls tested for preference between an estrous and non-estrous heifer using a test pen with sexually experienced bulls showing no preference (Geary et al., 1991). However, bulls prefer a pair of heifers engaging in female-female mounting over a pair of heifers unable to mount one another (Geary and Reeves, 1992). Perhaps a test with a

larger neutral zone and multiple females would be better suited to test for a preference in male goats.

For the trial consisting of a 5 min sexual performance test followed by a preference test, males could freely interact with the estrous and non-estrous females that would be used in the preference test. During the sexual performance tests males only mounted the estrous female. Rank of mounting frequency was negatively correlated with rank of preference for an estrous female indicating that the more the male had mounted the female, the less time he spent in close proximity to her during the preference test. As a promiscuous species, male goats have been selected to mate with many females in a given breeding season. There may be an advantage to losing interest in a female after mating, as this would contribute to the male seeking another potential mate. Perhaps if a different estrous female from the sexual performance test was used in the preference test, the males may have preferred the estrous female. Sexual activity was able to evoke a preference in the sexually experienced males in the Y-maze experiment, but the sexual performance tests were conducted with different females than those used as the stimulus females in the Y-maze. Also, in the Y-maze experiment the sexual performance and preference tests were conducted on different days, unlike the current experiment.

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CURRICULUM VITA

Andrea M. Haulenbeek

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| 2000 - 2004 | Rutgers University, Cook College, New Brunswick, NJ Bachelor of Science, Animal Sciences |
| 2004 - 2006 | Teaching Assistant, Department of Life Sciences Rutgers University, New Brunswick, NJ |
| 2006 - 2008 | Teaching Assistant, Department of Animal Sciences Rutgers University, New Brunswick, NJ |
| 2004 - 2009 | Rutgers University, The Graduate School - New Brunswick, New Brunswick, NJ Doctor of Philosophy, Endocrinology and Animal Biosciences |

Publications

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|------|---|
| 2004 | Sweeney, A. M. , Imwalle, D. B., Katz, L. S. Failure of the straight-arm runway to test sexual motivation in male goats. International Society for Applied Ethology North American Regional Meeting, West Lafayette, IN. |
| 2005 | Sweeney, A.M. , Katz, L. S. Use of a Y-maze to assess partner preference in male goats. 38 th Annual Meeting of the Society for the Study of Reproduction, Quebec, Canada. |
| 2006 | Haulenbeek, A. M. , Katz, L. S. Partner preference in male goats to measure sexual motivation. 10 th Annual Meeting of the Society for Behavioral Endocrinology, Pittsburgh, PA. |
| 2007 | Haulenbeek, A. M. , Katz, L. S. Female tail wagging elicits partner preference in male goats. 11 th Annual Meeting of the Society for Behavioral Endocrinology, Pacific Grove, CA. |
| 2008 | Haulenbeek, A. M. , Katz, L. S. Female tail wagging enhances sexual performance in male goats, <i>Capra hircus</i> . 45 th Annual Meeting of the Animal Behavior Society, Snowbird, UT. |
| 2009 | Haulenbeek, A. M. , Colendreo, A., Katz, L. S. Partner preference and sexual performance in male goats. 13 th Annual Meeting of the Society for Behavioral Endocrinology, East Lansing, MI. |