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Assessing Female Promiscuity from Behavioral Observation Beyond Male Influence in Saint Croix Ewes

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Abstract: To determine whether ewes would actively search for more than one ram to mate, 20 mature Saint Croix female sheep were induced into estrus and exposed to three adult Saint Croix rams attached by light chains to posts in each corner of a triangular field. During five days of continuous observation after progesterone withdrawal, the number of mounts and ejaculations received by each ewe and the number of rams involved was recorded. Every 15 min during the same 5 days, all ewes <10 m from the rams were listed. Thirty seven percent of the ewes were served exclusively by one ram, 43.7% by two and 18.75% received at least one ejaculation from each of the three males. After subtracting for the analysis those females that only mated once (n = 4), it was found that 16% of the ewes received two ejaculations, all from the same male, while 83% ($p<0.01$) received three or more serves from two or more different rams. Hence, ewes receiving more ejaculations sexually interacted with more rams ($r^2 = 0.72$; $p<0.05$). All ewes observed in close proximity with the rams were mounted. Ewes displayed sexual preference for one ram ($p<0.01$). Eighty seven, 50.0 and 43.75% of the ewes, were served by ram A-C, respectively. It was concluded that more than 80% of the ewes receiving three or more serves, actively search for more than one ram to mate and females receiving more ejaculations, interact with more males.

Key words: Promiscuous mating, proceptivity, ram-seeking behavior, sexual behavior, ejaculation, Saint Croix

INTRODUCTION

Promiscuous systems where both males and females mate several times with different individuals are widespread among ruminants (Gibson and Jewell, 1982). The *Ovis aries* is recognized as promiscuous specie with no evidence of the development of pair bonds between male and female (Lynch *et al*., 1992).

Ewes have been classified as promiscuous because they accept matings from several rams. However, it is unknown how active is their participation in promiscuity, or if they just remain in a passive receptivity allowing a number of copulations mostly determined by the sexual performance of the males (Price *et al*., 1996). In addition, most of the recent studies of sheep behavior have been orientated to the active role of the male rather than the more passive behavioral role of the ewe (Houpt, 1998).

Proceptive behavior is presumably of importance in determining promiscuity by the ewe. The proceptivity of ewes seems to consist of both affiliative behavior (Beach, 1976), which is the tendency to approach and remain in the vicinity of a ram and physical contact responses (Lindsay, 1996). Ram-seeking behavior in ewes can be very active (Fabre-Nys *et al*., 1997). For example, it is known that estrous ewes will seek out rams (Hafez, 1952), will approach and mate with tethered rams (Fletcher and Lindsay, 1968; Lindsay and Fletcher, 1972; Allison and Davis, 1976), can compete with other ewes for the attention of the male (Hulet *et al*., 1962; Tomkins and Bryant, 1972) and may, in some cases even initiate the sexual episode (Banks, 1964; Tomkins and Bryant, 1972).

Significant advances have been made in the behavioral study of rams in this mating system (Coltman *et al*., 1999; Preston *et al*., 2003, 2005). However, studies of the role of the ewe in promiscuity are scarce and there is almost no information available in hair sheep. The present study was designed to assess female promiscuity from behavioral observation trying to isolate male influence in Saint Croix sheep, determining whether ewes would actively search for more than one ram to mate, or if female promiscuity is the result of male active search for receptive ewes.

MATERIALS AND METHODS

Animals were 20 sexually experienced female Saint Croix sheep ranging 36-37 months of age at the time of the experiment, maintained at the University of the State of Morelos Mexico, 18°37'N and 99°19'W, located 899 m above sea level with an average annual rainfall of 800 mm and temperature of 23°C.

The ewes were induced to exhibit estrus by insertion of intra-vaginal sponges containing 30 mg fluorogestone acetate (Chronogest, Intervet, Mexico) for 12 days. After removal of sponges, ewes were injected im with 250 UI of pregnant mare serum gonadotrophin.

Blood samples were taken in days two and nine after sponge withdrawal. Samples were collected in evacuated glass tubes, by venipuncture of the jugular vein. Samples were immediately cooled and held in ice water until serum separation by centrifugation within 40 min of collection. The samples were frozen at -20°C, until analysis. Blood samples were taken for analysis of Progesterone (P_4) concentration using commercial kits (Pharmaceuticals, Diagnostic Division). The intra and inter-assay coefficients of variation were 7.42 and 6.28%, respectively. Serum P_4 content from the 1st and 2nd sample was compared to establish that ovulation had occurred. P_4 levels had to be <1 ng mL^{-1} in the first sample, followed by plasma progesterone levels >1 ng mL^{-1} 7 days later. According to P_4 concentrations, all animals responded to the synchronization treatment, ovulating within the observation period.

Three intact sexually experienced mature rams averaging 2-years-old were used as stimulus males in this study. These males were tethered by means of leather collars, to turnbuckles, a steel ring and 10 m fine link chains attached to steel posts in each corner of a triangular field of 50 m per side. In this situation the rams could mate only with ewes that showed ram-seeking activity. Rams were tethered for eight hours on each of three days prior to the experiment, to accustom them to restraint.

Animals were maintained in this 1,250 m^2-irrigated pasture of grass (*Cenchrus ciliaris* and *Cynodon* sp.) and white clover during 5 days of observation, where they received a concentrate of sorghum, molasses and chicken manure from a trough in the middle of the field, where a water source was also allocated. Concentrate and water were offered *at libitum* all time. Water and food were also available for the rams at their respective places, but limited to what they could eat and drink in one hour, to avoid the attraction of the ewes by these elements. Shade areas were allocated at the corners and at the middle of the field.

Large numbers were painted on the flanks of sheep to facilitate individual identification and direct continuous visual observation of the animals was carried out from at least two observers at a time, for periods of 4 h. Observations started at the time of sponge withdrawal and lasted for 5 days.

The observers recorded the identity of ewes that were mounted with and without an ejaculation, the number of ejaculations received and the number of rams involved. Also, every 15 min all females within 10 m from the rams were registered.

A Proportion test (Montgomery and Runger, 1986) was used to determine if ewes visited each ram equally; to compare the percentage of ewes mated by one ram vs. those receiving serves by two or more males from those ewes receiving more than one ejaculation and the proportion of ewes mated by each individual male. Pearson correlation coefficients were computed between the total number of serves received by each ewe and the number of different rams that mated with each female.

RESULTS

Eighty percent of the ewes (n = 16) received at least one ejaculation, while 15% were never observed in proximity with any ram and one ewe (5%) received mounts but was not served.

The total number of serves received by individual ewes throughout the 5 days of observation ranged from 0-13 (mean±SE = 4.2±1.9). Ewes receiving more ejaculations, sexually interacted with more rams ($r^2 = 0.72$; $p<0.05$; gl = 16; Fig. 1).

All ewes observed in close proximity (<10 m) with the rams were mounted. However, non-significant Pearson correlation coefficients were obtained between the total number of ejaculations received by each ewe and the total number of observations in which each ewe was in close proximity with a ram ($r^2 = 0.27$; $p>0.05$).

More ewes ($p<0.01$) were served by ram A (87%) in comparison with males B and C (50 and 43.8%, respectively; Fig. 2). Each circle illustrates the number of

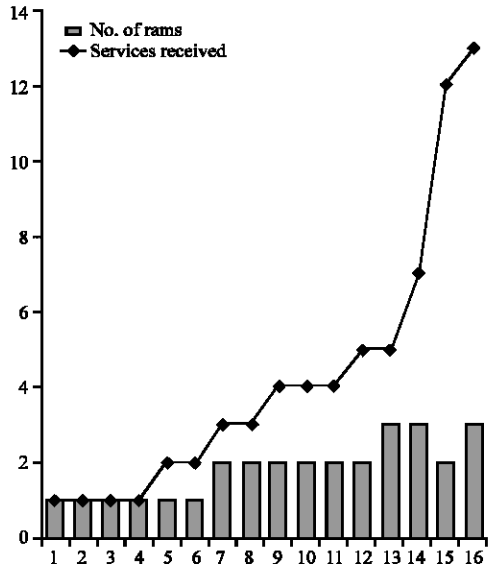

Fig. 1: Number of serves received by each ewe and number of rams that performed these ejaculations

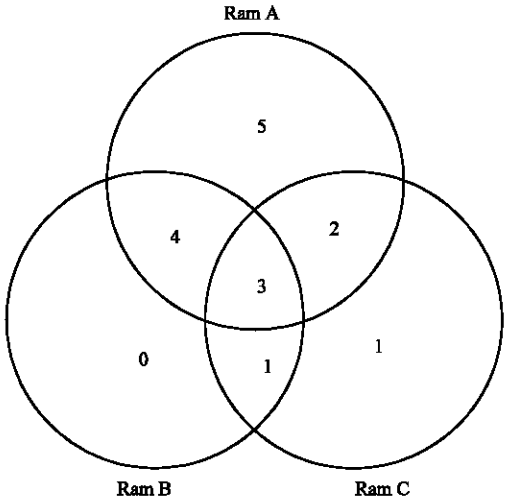

Fig. 2: Number of ewes mated (served) by an individual ram or in combination with other(s)

ewes mated by each ram, while circle overlaps represents ewes served by combinations of two or three males.

After subtracting for the analysis those animals that only mated once (n = 4), it was found that 16% of the ewes received two ejaculations, all from the same male, vs. 83% that received three or more serves from two or more different rams ($p<0.01$).

DISCUSSION

In theory, under random selection of rams, each male would have the same probability to mate (33%). However, the findings corroborate that the served ewes showed preferences for certain males, as 87% of the ewes mated with one specific ram. Result that is in agree with Kendrick *et al.* (1995) and Tilbrook *et al.* (1990) that reported that estrous ewes will choose a ram on a two-choice test and will do so even on the basis of a photograph. The observation that females displayed sexual selection among rams is not particularly novel. Allison and Davis (1976) observed that some individual rams within a breed appear particularly attractive and ewes seek the most active rams (Lindsay and Robinson, 1961). Furthermore, according to Estep *et al.* (1989), ewe preferences are strongly correlated with the rates of ram sexual solicitations.

Under unrestricted natural conditions of the field, mating preferences established by the males lead to an uneven distribution of serves among available estrous ewes and consequently some ewes are not mated at all (Synnott *et al.*, 1981). A similar situation may also occur in the present experiment due to the mating preferences displayed by the ewes.

According to the findings 83% of the ewes received three or more serves from two or more different rams, suggesting that more sexually active ewes would actively search for >1 ram to mate. It has been discussed earlier about the lack of independency in the probability of the number of ewes served by each ram or their combinations. The number of serves received by the ewes and the number of males involved are not distributed under random basis, due to the fact that each ejaculation received affects the occurrence of the next one (Lezama *et al.*, 2001). In addition, ewes been served by one male need to actively move away from that ram and search for a different male to get a second serve from other ram, representing a higher energetic cost shifting from one male to another (50 m according to the design of the present experiment) instead of remaining within the area of the first male selected.

The tendency for ewes to be mated by more than one ram reduced the impact of male dominance on conception rate. Furthermore, the number of ewes that are mated will influence the fertility of a flock and it has been observed that fertility increases with the number of times ewes are mated (Mattner and Baden, 1967; Cameron *et al.*, 1987) and by ewes being served by >1 ram (Knight and Lindsay, 1973; Allison, 1975; Allison and Davis, 1976).

The finding that not all ewes were mounted or seen in proximity of the rams is in agreement with previous findings. Matthews *et al.* (1991) observed that approximately, 75% of mature estrous ewes migrate towards and remain near tethered rams. Furthermore, Lindsay and Fletcher (1972) found that about 75% of intact ewes visited a tethered ram during a particular estrous period, observing a greater number of ewes showing estrous behavior than ram seeking activity. They also, found that at different estrogen doses, the number of spayed ewes showing estrous behavior was larger than those seeking rams.

Even though, a positive relationship between proceptivity and receptivity has been demonstrated by significant correlations between the measures of proceptivity and the number of mounts and/or ejaculations received by ewes (Tilbrook *et al.*, 1990) in the present study, a low correlation was observed between the time spent in close proximity with the rams and the number of ejaculations received. This results are in agreement with Price *et al.* (1996) and Tilbrook *et al.* (1987), suggesting that the soliciting behavior of the ewes had less influence on whether or not they are mated than ram's mating preferences.

Price *et al.* (1996) also found that rams that attained six ejaculations in a relatively short period (classified as high performers), mated fewer times with individual ewes than low-performing rams. As a consequence, high

performance rams tended to mate with a greater number of different ewes than did low performance rams. Similarly, under the conditions of the present experiment, we found that the more sexually active ewes achieved more serves from more individual males than did more passive females.

CONCLUSION

It was concluded that >80% of the ewes receiving three or more serves, actively search for >1 ram to mate and females receiving more ejaculations, interact with more males.

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