

Sexually inexperienced anestrus goats are able to exhibit sexual behaviours exposed to sexually active bucks

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Abstract The aim of this study was to determine whether sexually inexperienced females could display proceptivity and receptivity behaviours as the experienced, in the first exposure to males. Three groups of females (n=9 each) were used: i) sexually inexperienced, ii) with complete sexual experience, and iii) with limited sexual experience. Three male goats were subjected to photoperiodic treatment for 2.5 months of long days to stimulate their sexual activity during the natural sexual rest (March-April). During anestrus season, females were exposed to photo-stimulated males. Sexual behaviours were recorded during the first three days post-introduction of the males into female groups, in two daily periods of 20 min each. Sexually inexperienced females and those with complete sexual experience showed higher tail wagging than those with limited sexual experience ($P<0.001$). Sexually inexperienced females displayed higher female-female sniffing and emission of urine than those groups with complete and limited sexual experience ($P<0.001$ and $P<0.05$, respectively). Females with limited sexual experience displayed higher female-female mounts than those groups inexperienced and with complete sexual experience ($P<0.05$). In addition, females with complete sexual experience displayed higher female-male sniffing than those groups inexperienced and with limited sexual experience ($P<0.001$). Receptivity behaviour did not differ between female groups ($P>0.05$). We concluded that sexually inexperienced anestrus females display proceptivity and receptivity behaviours as those sexually experienced exposed to photo-stimulated males.

Keywords: anovulatory does, *Capra hircus*, male effect, photoperiod, seasonality

Introduction

Sexual activity in sheep and goat females is regulated by photoperiod (Duarte et al 2008; Abecia et al 2015). In these females the period of sexual activity is known as natural reproductive season, and another one of sexual inactivity is called seasonal anestrus (Delgado et al 1991, 1992; Restall 1992; Chemineau et al 2006; Duarte et al 2008). If sheep and goat females are exposed suddenly to a male during seasonal anestrus, in a relatively short time they exhibit sexual behaviours. This technique of sexual biostimulation is known as the male effect (Shelton 1960; Martin et al 1986; Chemineau 1987; Delgado et al 2009).

In the mammals, it is known that the females in sexual activity display stereotyped behaviours towards the male that are expressed in two motivational phases, and are known as proceptivity and receptivity (Beach 1976). During the proceptive phase, females display behaviours in order to attract the attention of the male, and initiate sexual contact with him; while in the receptive phase, females allow the consummation of the sexual encounter with the male (acceptance of mounts with intromission; Beach 1976; Price 1985; Fabre-Nys and Gelez 2007). For example, sexually active sow exhibits proceptivity behaviours when is in physical contact with the boar, in addition they display head-butting, and sniffs the flanks and ano-genital region of the boar (Hemsworth 1985). In comparison, in ewes during the same phase, they exhibit movements with the head towards to the male, remain close to him, and exhibit tail wagging (Gonyou 1991; Gelez and Fabre-Nys 2004; Gelez et al 2004). In sheep, sexually inexperienced females, i.e. those who have not had socio-sexual contact with males are less proceptive and receptive than those with sexual experience (Gelez et al 2004; Hawken et al 2008). In comparison, sexually experienced goats, i.e. when females have had socio-sexual contact with males, during natural or induced estrus, they exhibit proceptivity behaviours such as tail wagging and female-female mounts (Llewelyn et al 1993;

Imwalle and Katz 2004). In fact, sexually experienced anestrus female goats are more receptive when exposed to photo-stimulated males than non-photo-stimulated (Delgadillo et al 2002; Rivas-Muñoz et al 2007; Loya-Carrera et al 2014; Muñoz et al 2016).

To our knowledge, there are no studies in goats which have reported neither the effect of lack of sexual experience in females, nor the restriction of sexual experience, to display of proceptivity behaviours during seasonal anestrus when exposed to photo-stimulated males. Therefore, the hypothesis in the present study states that sexually inexperienced goats, during anestrus season, could display lower sexual behaviours in their first exposure to males, than sexually experienced goats. The objective of the present study was to determine whether sexually inexperienced females, in their first exposure to photo-stimulated males, could exhibit sexual behaviours of proceptivity and receptivity as those sexually experienced goats.

Materials and Methods

Ethical note

The management of the females and males in the present study was carried out following the protocol of the Norma Oficial Mexicana, according to specifications for the production, care and management of laboratory animals (SAGARPA 2001). The early separation of the kids from their mothers, and artificial breastfeeding that they received did not disrupt normal growth and development in these females.

Animals and description of experimental groups

Twenty-seven creole goat kids were born on January 10 ± 2 days (mean value \pm standard error of mean) from the Laguna region in the State of Coahuila (latitude $26^{\circ}23'N$ and longitude $104^{\circ}47'W$) during the natural breeding season were used. The goat kids were kept at all times in housing. The goat kids were separated from their mothers at 3 days of age, and were fed with goat's milk through an artificial breastfeeding system, which has shown have not negative effects on the development and weaning of goat kids (Lu and Potchoiba 1988; Luo et al 2000). In addition, were given forage and water *ad libitum*. The goat kids remained in these conditions until 40 days of age. Then, females were randomly distributed into three groups. The first, sexually inexperienced females ($n=9$) were kept isolated from males in a pen of 5×5 m. The second, with complete sexual experienced females ($n=9$) were maintained in complete permanent contact with two vasectomized males. These females and males were placed in a single pen (5×5 m). The third, with limited sexual experienced females ($n=9$) was

maintained in restricted permanent contact with two vasectomized males. Females were placed in a pen (5×5 m) adjacent to vasectomized males (5×2 m), separated by a division of wire-mesh. Vasectomized males were 2 years old on average. Females with complete and limited sexual experience remained in contact with vasectomized males since artificial breastfeeding was suspended (40 days old), until the next natural breeding season when females were 12 months old. Afterwards, the vasectomized males were removed from the pens. Females remained in these conditions until behavioural measurements were carried out.

Females were fed with alfalfa hay *ad libitum* (18% crude protein, 1.95 Mcal/kg) and commercial concentrate (18% crude protein; 2.05 Mcal/kg) according to their nutritional requirements since weaning until the end of the study. Vasectomized males were fed with alfalfa hay *ad libitum* (18% crude protein; 1.95 Mcal/kg) and 300 g of commercial concentrate (14% crude protein, 2.5 Mcal/kg). For females and males drinking water and mineral salts (12% phosphorus and 11% calcium) were always available.

Treatment of artificial photoperiod applied to males

In order to induce an increase in sexual behaviour, odor, vocalizations and sperm production in males during the natural sexual rest (March-April; Delgadillo et al 2002), a treatment of artificial photoperiod was applied. Entire males ($n=3$) of 3 years of age on average were used. Males were housed in outdoor pen (5×5 m) in order to receive the treatment of artificial photoperiod (with lamps of 65 W). Light intensity was at least 300 lx at the level of the eyes of the males (Figure 1). The treatment of long days applied to the males was for 2.5 months (16 h of light/day), starting on November 1 to January 15. From January 16 the males perceived the natural photoperiod.

Preparation of females

In goats of this region, the seasonal anestrus is from March to September (Duarte et al 2008). Therefore, in the month of March, when females were 14 months old, they were submitted to an evaluation to determine their anovulatory status. The anovulation was determined with the help of studies of ultrasonography, and was resolved when corpora lutea were not detected. The ultrasonography studies were performed on March 10 and 20 before introducing photo-stimulated males into three groups of females. The ultrasound used was an Aloka SSD-500 (Tokyo, Japan) with a 7.5 MHz transducer. In addition, three days before beginning the experiment, and based on the scale described by Walkden-Brown et al (1997), body condition score was measured in sexually experienced females, with complete

and limited sexual experience (2.6 ± 0.08 , 2.5 ± 0.09 and 2.5 ± 0.07 , respectively; $P > 0.05$).



Figure 1 Males were submitted to a treatment of artificial long days, light intensity was at least 300 lx at the level lateral of the eyes of the males. The treatment of artificial long days applied to the males was for 2.5 months (16 h of light/day) from November 1 to January 15.

Male effect

On March 25 (day 0; 08:00 h), a photo-stimulated male was placed in contact with each group of females (sexually inexperienced, with completed and limited sexual experience). Afterwards, the males were exchanged in each group of females in the morning (08:00) and in the afternoon (18:00). This management was maintained for 15 days.

Behavioural measurements

For males, behavioural measure were performed with focal continued observation (Martin and Bateson 2007), on days 0, 1 and 2, for a period of 20 min (08:00 to 08:20 and 18:00 to 18:20). Individual total number of occurrences of ano-genital sniffing, nudging, self-urination, flehmen response, mounting attempts, mounts without and with intromission were recorded to verify that males were sexually active. During behavioural measure all males in contact with the three groups of females displayed sexual activity (Table 1; Figure 2 and Figure 3; Flores et al 2000; Loya-Carrera et al 2014).

Regarding to females we decided that measurements should be taken only during the first three days post-introduction of males into female groups, since it has been demonstrated to be the time lapse sufficient for photo-stimulated males induce sexual response in anestrous females through the male effect (Flores et al 2000; Rivas-Muñoz et al 2007; Bedos et al 2010). So that females were observed with a similar method as the described for the males, and the frequency of the next proceptive behaviours were recorded: tail wagging, female-female sniffing, female-male sniffing, female-female mounts, and emission of urine (Table 1). Receptive sexual behaviour registered was acceptance of the mount with intromission (Table 1). A female was considered as receptive when she remained immobile and accepted to be mounted by the male (Chemineau et al 1992). In addition, frequency of vocalizations (high bleating) was recorded. The observations were videotaped using a video camera (SONY-V8, Japan). The video-recordings were analyzed later in the laboratory using the program Observer Video Pro version 4.0 (Noduls, The Netherlands).

Table 1 Sexual behaviours in male goats in contact with females.

Behaviour	Description
Male	
Nudging	He approaches the female sometimes vocalizing and lowering his head
Ano-genital sniffing	He approaches his nose near the female (<0.005 m) smelling her genital or anal areas
Mounting attempts	He attempts to mount the female, but does not place its body on top
Flehmen response	After smelling and/or tasting the females' urine he lifts its head and upper the lip up
Self-urination	He urinates himself by turning his penis onto his face
Mounts without and with intromission	He mounts the female either penetrating or not penetrating her
Female	
Tail wagging	She moves its tail quickly sideways. The frequency is measured by the number of episode in a time unit
Female-female sniffing	When a female approaches another female in order to smell a part of her body
Female-female mounts	A female climbs up getting on top of another female
Emission of urine	She does urinate
Acceptance of mounts with intromission	A female stays still, allowing to be mounted and penetrated by a male

Statistical analysis

The total frequency of behaviors as tail wagging, female-female sniffing, female-male sniffing, female-female mounts, acceptance of mounts, vocalizations, and emission of urine, were compared between groups of females using the Chi-square test for multiple group comparison and with the Fisher exact probability test. Statistical analysis was carried out using the statistical package SYSTAT version 13.00.05 (Systat Software, Inc., Chicago, IL, USA).

Results

Proceptive behaviours

Sexually inexperienced females and the group with complete sexual experience displayed higher frequency of tail wagging than those with limited sexual experience ($P < 0.001$, Figure 4). Likewise, sexually inexperienced females displayed higher female-female sniffing ($P < 0.001$; Figure 4) than those groups with complete and limited sexual experience. Furthermore, sexually inexperienced females

displayed higher emission of urine ($P < 0.05$; Figure 4) than those groups with complete and limited sexual experience. In contrast, the group with limited sexual experience displayed higher female-female mounts ($P < 0.05$; Figure 4) than those groups inexperienced and with complete sexual experience. In addition, the group with complete sexual experience displayed higher female-male sniffing ($P < 0.001$; Figure 4) than those groups inexperienced and with limited sexual experience.

Receptive behaviour

The frequency of acceptance of the mounts did not differ ($P > 0.05$; Figure 4 and Figure 5) between the three groups of females.

Other behaviour

Sexually inexperienced females emitted higher vocalizations ($P < 0.001$; Figure 4) than those groups with complete and limited sexual experience.

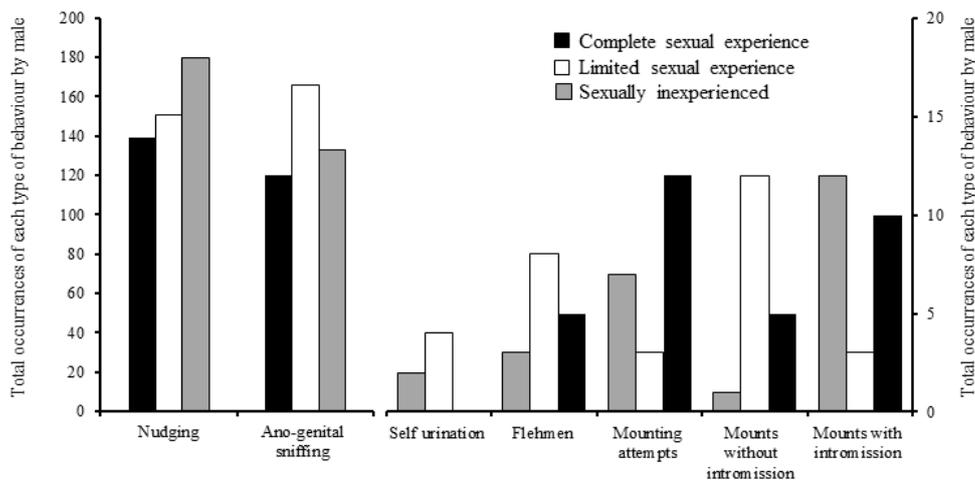


Figure 2 Individual occurrences of nudging, ano-genital sniffing, self-urination, flehmen response, mounting attempts, mounts without and with intromission in males interacting with sexually inexperienced females (gray bars), with complete sexual experienced females (dark bars) and with limited sexual experienced females (white bars). Sexual behaviour of males was observed from 08:00 to 08:20 and 18:00 to 18:20 on days 0, 1, and 2 after introduction of males in the groups of females. Males were rendered sexually active by exposure to artificial long days (16 h of light by day) from November 1 to January 15.

Discussion

The results of the present study indicate that sexually inexperienced anestrus goats were able to display proceptive and receptive sexual behaviours, as those sexually experienced goats, when they were exposed for the first time to photo-stimulated males. We considered in our study that in spite of the fact that sexually inexperienced females were exposed to a photo-stimulated male suddenly at 14 months of age, lack of sexual experience or familiarity to males did not

prevent them to display sexual behaviours. In fact, sexual behaviour was similar between sexually inexperienced goats, and in those were already familiarized with the male completely or restricted since weaning. Our results also indicate that lack of sexual experience did not affect the response to the male effect during seasonal anestrus. This is relevant because it indicates that there are innate factors involved in the acceptance of mounts (receptivity) which are activated when females receive sensorial stimuli, in this case sexual bio-stimulation by photo-stimulated males (Pfaus et al

2001; Delgadillo et al 2002). Then, when females exhibit proceptive behaviours learning factors are involved and facilitates mating (Agmo 1999; Pfaus et al 2001; Gelez et al 2004).

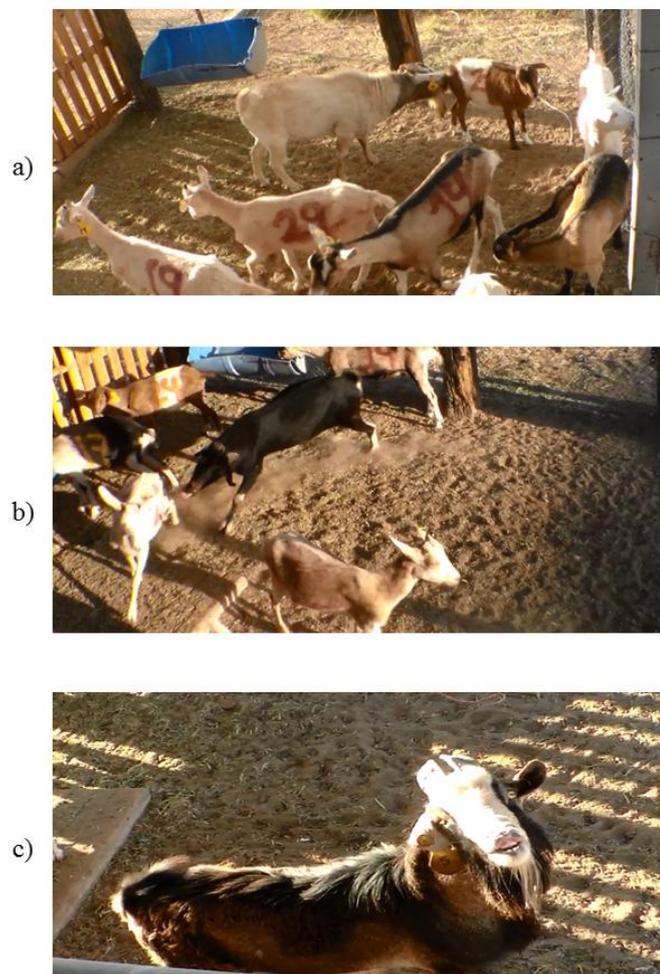


Figure 3 Sexual behaviours of a) ano-genital sniffing, b) nudging, and c) flehmen response exhibited by photo-stimulated males interacting with anestrus females. Males were rendered sexually active by exposure to artificial long days (16 h of light by day) from November 1 to January 15.

In the present study, sexual behaviour of tail wagging was more frequent in the groups with sexual inexperience and complete sexual experience than in the restricted females group. These results are in accordance with literature, because tail wagging also was observed in ewes, and did not differ between sexually inexperienced and experienced anestrus females (Hawken et al 2008). In addition, these behaviour also was observed in goats, during the proceptive phase, that were in induced estrus (Imwalle and Katz 2004; Haulenbeek and Katz 2011), and in females that were naturally in estrus, displaying not only tail wagging but also remaining close to the males (Llewelyn et al 1993). According to our results and those studies mentioned above, it is shown that tail wagging is a characteristic sign of estrus in both sheep and goats. Therefore, ewe and goat females

display tail wagging as a visual signal to establish communication with the males.

The female-female sniffing was more observed in sexually inexperienced goats than in complete and limited sexual experience female groups. Perhaps this response was due to fact that sexually inexperienced goats were more familiarized with other females than males, and then they re-directed such behaviour, as has been demonstrated in male goats (Ungerfeld et al 2014).

The homosexual behaviours, i.e. when males exhibit sexual behaviours to individuals of the same sex, are more frequent in males that had been isolated from females (Ungerfeld et al 2014). In our study, anestrus females with limited sexual experience exhibited more female-female mounts than sexually inexperienced goats and those with complete sexual experience, which could be associated with an attempt to attract attention of the male, and initiate a sexual interaction, perhaps caused by previous restriction to males (Beach 1976; Shearer and Katz 2006). Llewelyn et al (1993) found that behaviour of female-female mounts was exhibited by goats with higher social rank which were in estrus during the natural breeding season also in order to attract attention of males.

In regards to female-male sniffing, females with complete sexual experience exhibited more often this behaviour than sexually inexperienced and those with limited sexual experience. This response was probably due to the fact that females already had sexual experience and identified easily the presence of males.

The receptive behaviour (acceptance of mounts) did not differ between the three groups of goats exposed to photo-stimulated males. In addition, this response was similar to that reported in sexually inexperienced goats (isolated from males) and in sexually experienced goats without sexual intromission before the male effect (Fernández et al 2011). Likewise, receptivity exhibited by sexually inexperienced females during their first exposure to photo-stimulated males was similar to exhibited by females with previous sexual experience, and exposed to photo-stimulated males (Rivas-Muñoz et al 2007; Luna-Orozco et al 2008; Loya-Carrera et al 2014).

In contrast, our results differed from those of Delgadillo et al (2012), where sexually inexperienced females and exposed for the first time to vocalizations of males, displayed lower acceptance of mounts than those experienced. These results suggest that total contact with males is necessary for females to exhibit sexual behaviours (Delgadillo et al 2012). In our study we found that sexually inexperienced females emitted more vocalizations and emissions of urine than other two groups of goats. This response was probably due to the fact that females suffered stress when they were placed in contact with the males for the first time, as was observed in ewes (Gelez et al 2004).

Furthermore, the emissions of urine can also be considered as proceptivity behaviour as was demonstrated in mice (*Mus musculus*, Dizinno et al 1978). Also it has been shown that critical situations such as exposure to new stimuli (Chojnacki et al 2014), social isolation (Terrazas et al 2012) and hunger

induce an increase in vocalizations, and eliminations (Poindron et al 2007). It has also been shown that goat females are more sensitive to respond that way than males (Terrazas et al 2012; Chojnacki et al 2014).

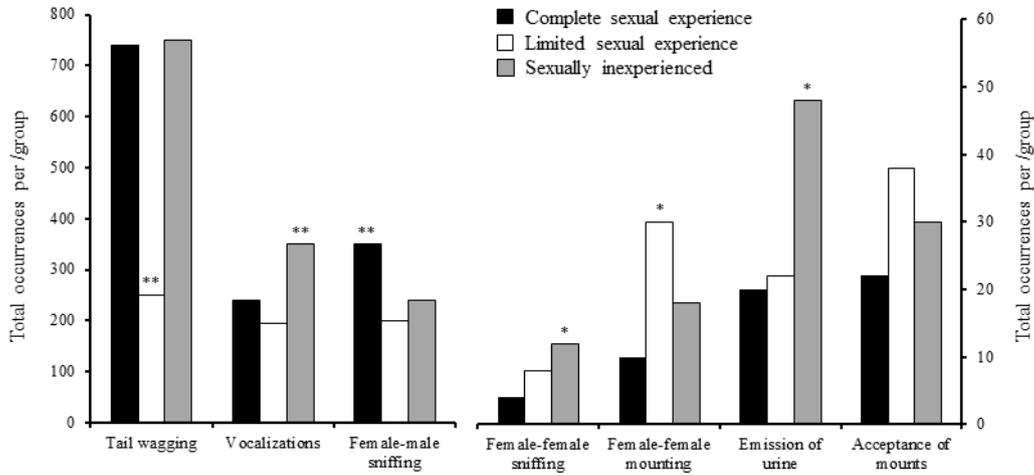


Figure 4 Frequencies of tail wagging, vocalizations, female-male sniffing, female-female sniffing, female-female mounts, emission of urine, and acceptance of mounts in sexually inexperienced females (gray bars), with complete sexual experienced females (dark bars), and with limited sexual experienced females (white bars). Sexual behaviour of females was observed from 08:00 to 08:20 and 18:00 to 18:20 on days 0, 1 and 2 after introduction of males in the groups of females. Males were rendered sexually active by exposure to artificial long days (16 h of light by day) from November 1 to January 15. *(P<0.05), **(P<0.001).

In the present study, our females deprived of the male presence before the male effect displayed sexual behaviours as those with previous experience. This response was due to the fact that females were exposed to males that exhibited high sexual behaviour. It is reported in literature that photo-stimulated males which exhibit a high level of sexual behaviour as nudging, ano-genital sniffing, self-urination, flehmen response, mounting attempts, and mounts with intromission are efficient for inducing sexual response in anestrus females. On the other hand, those males with low

sexual behavior are inefficient to induce sexual response in anestrus females (Delgado et al 2002; Muñoz et al 2016). In addition, photo-stimulated males exhibiting sexual behaviour during natural sexual rest inducing a high percentage (>92%) of estrus (receptivity) in sexually experienced females (Rivas-Muñoz et al 2007; Luna-Orozco et al 2008). Finally, the results mentioned above suggest that photo-stimulated males first induce the proceptive phase, and then the receptive phase (acceptance of mounts) in anestrus females.



Figure 5 Receptivity sexual (acceptance of the mount) in sexually inexperienced females exposed to photo-stimulated males. Males were rendered sexually active by exposure to artificial long days (16 h of light by day) from November 1 to January 15.

Conclusions

Sexually inexperienced females during anestrus season display proceptivity and receptivity behaviours as those sexually experienced, when exposed to photo-stimulated males for the first time through the male effect.

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