Absence of previous sexual experience did not modify the response of anoestrous goats to photo-stimulated bucks in Spring

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ABSTRACT
Sexually inexperienced rams display lower frequency of nudging, ano-genital sniffing, mounting attempts, and mounts with intromission than experienced ones. Experienced photo-stimulated males display high level of sexual behaviour. The aim of this study was to determine: i) whether sexually inexperienced photo-stimulated males display similar sexual behaviour as the experienced ones, and ii) whether these inexperienced bucks can stimulate sexual response in anoestrous goats, as the experienced ones. Sexually inexperienced and experienced photo-stimulated males (n = 5 each) were exposed to these males for 15 d. Male sexual behaviour was registered for three days, upon their introduction into female groups. On day 1, the inexperienced males displayed higher nudging than the experienced ones (p < .001), whereas on days 2 and 3, the experienced males displayed higher nudging than the inexperienced ones (p < .001). The ano-genital sniffing, mounting attempts, and mounts with intromission did not differ between the inexperienced and experienced groups (p > .59). The proportion of females ovulating did not differ between the two groups (82%; p = 1.0). Pregnancy rate (68%; p = .829) did not differ between the females exposed to inexperienced or experienced males. It is concluded that sexually inexperienced photo-stimulated males show similar sexual behaviour as the experienced ones, and they are able to induce sexual response in seasonally anoestrous goats through the male effect.

Introduction
Sexually inexperienced males, i.e. those who have never had any sexual interaction with females, display lower frequency of sexual behaviour as nudging, flehmen, ano-genital sniffing and mounting with intromission than experienced ones. When such inexperienced males are exposed to females for the first time, they exhibit less sexual behaviour; examples of such behaviour include kick and flehmen in stallions (McDonell 1986), flehmen in bulls and boars (Hemsworth et al. 1977; Price 1985), nudging, flehmen, ano-genital sniffing and mounting in male goats and rams (Price and Smith 1984; Simitzis et al. 2006). Moreover, when inexperienced rams are exposed to ewes, the onset of sexual behaviour of these males is delayed by several days and a considerable fraction (30%) of them remains sexually inactive. Besides, a similar percentage of rams show sexual inactivity, when reared within a unisexual group (Katz et al. 1988; Price et al. 1991, 1994). It is known that rams which display less mounts with intromission, are relatively inefficient for inducing oestrus and ovulations (Perkins and Fitzgerald 1994; Ungerfeld et al. 2008). In contrast, sexually experienced photo-stimulated males, which display high levels of sexual behaviour such as nudging, ano-genital sniffing, flehmen, mounting attempts, self-urination, and mounts with intromission, can efficiently stimulate sexual activity in anoestrous females (Flores et al. 2000; Delgadillo et al. 2002; Muñoz et al. 2016). Previous studies have indicated that sexually experienced males display high levels of sexual behaviour when they are subjected to a photoperiodic treatment of long days for 2.5 months (Delgadillo et al. 2002). In males, photoperiodic treatment increases the testosterone secretion, odour, and sexual behaviour during March and April, months that are considered a part of their sexual rest season (Delgadillo et al. 2002; Bedos et al. 2012). When sexually experienced photo-stimulated males are exposed to anoestrous goats, they can
Oestrus was induced in these females using two doses of oestradiol cypionate IM. The first dose (4 mg) was administered 24 h before they were placed in contact with the males, and the second dose (2 mg) was 48 h later. With this treatment, the females displayed oestrous behaviour for 5 d. Each male mounted and penetrated one female at least three times.

### Materials and methods

#### Animals, management and treatments

Ten male creole local goats (*Capra hircus*) were used. These males were born in December in the Laguna region, State of Coahuila, Mexico (latitude 26°23′N, longitude 104°47′W, altitude 1200 m above the sea level), 16 months before the beginning of the experiment. The Laguna region has a semi-arid climate (BSh), and an average annual rainfall of 266 mm (range: 163–504 mm) from June to September. The average annual relative humidity is 40.7%. Mean annual maximum and minimum temperatures are 36.6 °C between May and August and 5.7 °C between December and January, respectively. These goat kids were separated from their mothers at 3 d of age, and were artificially fed goat milk until they were 30 days old. They were provided with alfalfa hay (18% CP, 1.95 Mcal/kg) and commercial concentrate (14% CP, 2.5 Mcal/kg) according to their requirements, from 30 d of age until the end of the study. Water and mineral salts were given *ad libitum*.

When the goat kids were 31 days old, they were assigned randomly to one of two groups (n = 5 each). The first group (sexually inexperienced males; 10.3 ± 0.58 kg) was isolated from females. The second group (sexually experienced males; 9.5 ± 0.62 kg; *p > 0.05*) had visual, auditory, olfactory and restricted tactile contact with females through a wire-mesh. In October, during the natural breeding season, when the males were 10 months old, the second group (sexually experienced males) was placed in full physical contact with three females in oestrus, in order for them to acquire sexual experience through mating. Oestrus was induced in these females using two doses of oestradiol cypionate IM. The first dose (4 mg) was administered 24 h before they were placed in contact with the males, and the second dose (2 mg) was 48 h later. With this treatment, the females displayed oestrous behaviour for 5 d. Each male mounted and penetrated one female at least three times.

#### Photoperiodic treatment

When the males reached 11 months of age, both groups were placed in two outdoor pens (5 m × 4 m) and exposed to artificial long days (16 h of light per day) for 2.5 months, starting on November 1 (Delgadillo et al. 2002). To avoid contact between the inexperienced and experienced photo-stimulated males, the pens were separated by a distance of more than 2 km. During the experiment, when the males were placed in contact with females, the body condition score (BCS) of the males was determined by palpating their lumbar region. A scale of 1–4 was used for measuring the BCS, where 1 = emaciated and 4 = fat (Walkden-Brown et al. 1997); for the inexperienced and experienced photo-stimulated males, the scores were 3.2 ± 0.1 and 3.0 ± 0, respectively (mean ± SEM; *p > 0.05*).

#### Preparation of females

One hundred creole local females were used, which were 3–4 years old. During the non-breeding season the females were divided into two groups (n = 50 each) that were balanced according to their average BCS (1.8 ± 0.03 and 1.9 ± 0.03; *p > 0.05*), using the same method as described above. Females were fed 2.5 kg of alfalfa hay (18% CP, 1.95 Mcal/kg) with 200 g of commercial concentrate (14% CP, 1.7 Mcal/kg), and they had free access to water and mineral salts. The anovulatory status of females was determined by the absence of corpora lutea, using transrectal ultrasound examinations performed twice in March, 20 and 10 d before introducing the males into female groups. The transrectal ultrasound was performed using an Aloka SSD-500 (Tokyo, Japan) device equipped with a 7.5 MHz transducer. All the females were manually milked once daily (in the morning) during the study. Three days before introducing the males, each group of 50 anovulatory females was divided into 5 subgroups of 10 females each (allocated in open pens of size 4 m × 3 m).

#### Male effect

The two groups of females were separated by more than 200 m to avoid any possibility of interference
between males. On 1 April (Day 1; 0800 h), inexperienced and experienced photo-stimulated males were put randomly with each subgroup of females (1:10 ratio). Every morning for 15 consecutive days the males were switched among subgroups of females.

**Measurements**

Sexual behaviour of the photo-stimulated males was observed on days 1–3, after their introduction into female groups. The male sexual behaviour was documented from 08:00 to 09:00 h. Trained observers followed each of the males individually and recorded the sexual behaviour such as nudging, ano-genital sniffing, mounting attempts and mounts with intromission (Flores et al. 2000).

Ovulatory response and ovulation rates were assessed by the presence and the number of corpora lutea observed in each female using transrectal ultrasonography as mentioned above, 18 d after the introduction of the bucks. In goats, the use of transrectal ultrasonography has demonstrated to be a reliable technique for determining ovulations and ovulation rate induced by the male (Ginther and Kot 1994; Simões et al. 2007; Bedos et al. 2012). Pregnancy rates (pregnant does/does exposed to males) were determined by performing abdominal ultrasonography in the females, 50 d after the introduction of males, using the earlier-mentioned equipment, this time connected to a 3.5 MHz abdominal probe.

**Statistical analyses**

The males’ sexual behaviour was analysed using the Chi-square test for goodness of fit, considering a random distribution of 50% in each group, comparing the experienced and inexperienced photo-stimulated males. The Chi-square test was used for multiple-group comparisons and Fisher’s exact probability test was used for two-group comparisons to assess the statistical differences in the proportions of females that ovulated and the pregnancy rates. Ovulation rates were compared using the Kruskall–Wallis test followed by a Mann–Whitney U test. Data are expressed as mean ± standard error of the mean. Analyses were carried out using the statistical package SYSTAT 13 (2009).

**Ethical note**

This study complies with the procedures reported in the Official Mexican Rule that specifies the technical specifications for the production, care, and use of laboratory animals (SAGARPA 2001). The early separation of goat kids from their mothers as well as the artificial feeding did not affect their normal growth and development.

**Results**

**Sexual behaviour of sexually inexperienced and experienced males**

On day 1, the inexperienced males displayed higher occurrences of nudging than the experienced ones ($p < .001$; Figure 1). However, on days 2 and 3, the experienced males displayed more instances of nudging than the inexperienced ones ($p < .001$; Figure 1). On days 1–3, the occurrences of ano-genital sniffing, mounting attempts, and mounts with intromission did not differ between the inexperienced and experienced males ($p > .05$; Figure 1).

**Sexual response of females to sexually inexperienced and experienced males**

The percentage of females that ovulated did not differ between those in contact with sexually inexperienced or experienced males ($p = 1.0$; Table 1). In fact, more than 80% of the goats ovulated when exposed to either sexually inexperienced or experienced males (Table 1). Furthermore, ovulation rate did not differ between the females exposed to sexually inexperienced or experienced males ($p = .154$; Table 1). The pregnancy rate did not differ between the females that were in contact with sexually inexperienced or experienced males ($p = .829$; Table 1).

**Discussion**

The results indicate that sexually inexperienced photo-stimulated males showed similar frequency of sexual behaviour such as nudging, ano-genital sniffing, mounting attempts, and mounts with intromission as the experienced males, when exposed to anoestrous female goats. The current results clearly demonstrate that since day 1, when the inexperienced males were exposed to anoestrous females for the first time, 100% of them exhibited sexual behaviour such as nudging, ano-genital sniffing, mounting attempts, and mounts with intromission. In this study, both inexperienced and experienced males were rendered sexually active by exposure to 2.5 months of artificial long days; as mentioned earlier, this treatment enhances sexual behaviour, odour, and vocalisations during their natural sexual resting period (March and April; Delgadillo et al. 2002). Similarly, the level of sexual
behaviour displayed by inexperienced males has been shown to be similar to that of sexually experienced photo-stimulated males exposed to anoestrous females through the male effect (Bedos et al. 2010, 2012; Loya-Carrera et al. 2014). However, in the present study, the occurrences of nudging showed changes between the male groups during the three days of measurement. On the first day, inexperienced males displayed greater occurrences of nudging than the experienced ones. In contrast, on days 2 and 3, the experienced males displayed more nudging than the inexperienced ones. This change was probably due to the natural variation in sexual behaviour found in animals (Katz 2008). Similarly, Bedos et al. (2016) found high variability in the sexual behaviour among males of the same breed and reproductive season, which received photoperiodic treatment and were exposed to anoestrous goats. In this study, the probable cause of such variability was due to the sexually inexperienced males having been isolated from females since weaning. Perhaps exposure to a new stimulus, in this case the females, altered their sexual behaviour expression. Despite this variation, the inexperienced males showed interest in anoestrous females since the first day. Therefore, the present findings differ from the results by sheep species that indicate that a considerable percentage of inexperienced rams remained sexually inactive (30 %) during the first day of contact with females; some of these rams show a lack of interest in females or a delayed onset of sexual behaviour (Katz et al. 1988; Price et al. 1988, 1991, 1994). Similarly, the results of this study differ from the results that showed that sexually inexperienced young rams (14–15 months of age) were unable to identify receptive females during the first contact between both sexes (Orgeur 1991).

In this study, the ovulatory and reproductive activity did not differ among females exposed to inexperienced and experienced males. In fact, for the females that ovulated, the ovulation rates and pregnancy rates were similar to the sexual and reproductive responses observed in anoestrous females exposed to sexually

Table 1. Ovulatory response and pregnancy rate in anoestrous female goats exposed to sexually inexperienced and experienced photo-stimulated males. Male goats were rendered sexually active during the non-breeding season by exposure to 2.5 months of long days (16 h of light per day). Differences between groups were not significant (ns).

| Females exposed to males sexually | Ovulations, % | Ovulation rate (mean ± SEM) | Pregnancy rate, % |
|----------------------------------|--------------|-----------------------------|-------------------|
| Inexperienced                    | 82           | 1.6 ± 0.08                  | 70                |
| Experienced                      | 83           | 1.5 ± 0.08                  | 68                |
experienced photo-stimulated males (Fernández et al. 2011; Bedos et al. 2012; Loya-Carrera et al. 2014). The present results differ from those reported in sheep, since young rams with lower sexual experience induced lower proportions of oovulations than adults (61 and 78%, respectively; Ungerfeld et al. 2008).

In fact, sexually inexperienced and experienced photo-stimulated males were separated from weaning until the end of the male effect as above mentioned, therefore inexperienced males did not see, sniff or hear the experienced ones. In contrast, previous studies show that stallions, boars and beef bulls improve sexual behaviour when observe mounts and mounts with intromission displayed by other males (Pickett et al. 1977; Hemsworth and Galloway 1979; Blockey de BMA. 1981). Thus in this study the photoperiod treatment was as effective in the inexperienced maintained in total isolation as in the experienced ones.

Therefore, this study strongly supports the importance of sexual behaviour displayed by sexually inexperienced photo-stimulated males, for inducing sexual activity in anoestrous female goats, as reported in previous studies (Delgadillo et al. 2006; Martínez-Alfaro et al. 2014).

**Conclusions**

Sexually inexperienced photo-stimulated males show similar sexual behaviour as the experienced ones, and they are able to induce sexual response in seasonally anoestrous goats through the male effect.

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**Disclosure statement**

No potential conflict of interest was reported by the authors.

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