Photoperiodic treatment of bucks markedly improves the response of seasonally anovulatory goats to the "male effect"

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RÉSUMÉ

Dans la région subtropicale du nord du Mexique, les races caprines locales montrent d'importantes variations saisonnières de la reproduction. Les femelles présentent une période d'anoestrus de mars à août, et les mâles sont en repos sexuel de janvier à avril. Ces variations sont contrôlées par les variations saisonnières de photopériode. L'activité sexuelle chez les femelles en anoestrus peut être induite par un effet mâle seulement juste avant le début spontané de l'activité sexuelle saisonnière. L'inefficacité de l'effet mâle à d'autres moments peut être due à une incapacité des femelles à répondre ou à une stimulation insuffisante de la part des mâles, qui sont aussi en repos sexuel. Pour vérifier ce point, deux études ont été effectuées pour déterminer si l'abscence de réponse de chèvres en anoestrus saisonnier à l'effet mâle est due à une faible stimulation par les mâles. Dans la première étude, 4 mâles (sexuellement inactifs; SI; n=4) ont été soumis aux variations naturelles de la photopériode, et 4 autres à 2,5 mois de jours longs à partir du 1er novembre, suivis de la pose de 2 implants de mélatonine (sexuellement actifs; SA; n= 4). Le 15 mars, des femelles anovulatoires de 2 troupeaux ont été mises en contact avec les mâles SI ou SA. Le comportement sexuel des mâles SA (approches latérales, tentatives de montes et montes) a été significativement supérieur à celui des mâles SI. Seulement 2/34 femelles avec les mâles SI ont ovulé et aucune n'a été détectée en oestrus durant les 35 jours d'observation. En revanche, toutes les femelles (40/40) avec les mâles SA ont ovulé et manifesté au moins un oestrus dans les 11 premiers jours après l'introduction des mâles. Afin de déterminer si la réponse obtenue n'était pas due à un effet troupeau, l'année suivante des femelles anovulatoires d'un même troupeau ont été utilisées. Là encore le comportement des mâles SA a été supérieur à celui des mâles SI. Une femelle sur 33 avec les mâles SI est venue en oestrus, contre 27/33 chez celles avec les mâles SA. En conclusion, l'absence de réponse à l'effet mâle n'est pas due à un état réfractaire des chèvres, mais plutôt à une faible stimulation de la part des mâles.

SUMMARY

In subtropical Northern Mexico, local goats show important seasonal variations of their sexual activity. In females, anestrus periods occur from March to August, while in males, the non-breeding season lasts from January to April. In this breed, seasonality is provoked by photoperiodic changes. The sexual activity of females can be induced during the anoestrous period by the male effect only before the onset of natural seasonal breeding. The failure of the male effect in other periods may be due to an inability of females to respond or to an inadequate stimulation from the males which are also in sexual rest. To clarify this point, two experiments were carried out to determine whether the inefficiency of the male effect during seasonal anestrus in goats is due to poor stimulating ability of the male. In the first study, one of two groups of males (Sexually inactive; SI; n = 4) remained under natural photoperiod while the other (Sexually active; SA; n = 4) was subjected to 2.5 months of long days from November 1, followed by the insertion of 2 subcutaneous implants of melatonin. On March 15, anovulatory females of 2 different flocks were exposed to either SI or SA bucks. The sexual behavior of SA males (nudging, mounting attempts and mounts) was significantly higher than that of SI males. Only 2/34 goats with SI males ovulated and none showed estrus behavior during the 35 days of study. In contrast, all females (40/40) with SA males ovulated and showed at least one estrus during the first 11 days following male introduction ($P < 0.001$). One year later, anovulatory females of a single flock were used to verify a possible difference of responsiveness between flocks. Again, the sexual behavior of SA males was higher than that of SI males and 1/33 goats showed estrus behavior in the SI-untreated group versus 27/33 in the SA-treated group over the first 14 days ($P < 0.001$). Therefore, the absence of response to teasing at this time of the year is not due to female unresponsiveness, but to insufficient stimulation from the male.

INTRODUCTION

Local breeds of goats and sheep originating from or adapted to subtropical conditions exhibit seasonality in their reproductive activity (Walkden-Brown et al., 1994; Pérez-Clariget et al., 1998; Delgadillo et al., 1999; Duarte et al., 1999). This seasonality is similar to that observed in breeds from temperate latitudes, in which sexual activity throughout the year is controlled by large changes of photoperiod (Lincoln and Short, 1980; Delgadillo et al., 1993, Malpaux et al., 1999). However, because the amplitude of photoperiod changes is moderate in tropical and subtropical latitudes compared with those of temperate areas, it is often thought that nutritional conditions are the main modulator of sexual activity in the tropics and subtropics (Kawas et al., 1992; Bronson and Heideman, 1994; Delgadillo and Malpaux 1996). For instance, in male Australian Cashmere goats raised in a subtropical environment, gonadal activity is mainly influenced by nutritional levels (Walkden-Brown et al., 1994). In subtropical Northern Mexico, a clear seasonal pattern of parturition in females under extensive conditions is observed, with a peak between November and February (Hoyos et al., 1991), suggesting increased sexual activity in June and July. In males, a season of sexual rest has been also described during the winter and spring (Delgadillo et al., 1997).

Regardless of its cause, reproductive seasonality represents a strong constraint to the farmers because milk and meat production also become seasonal. Indeed, the highly seasonal distribution of kidding induces a seasonal availability of meat and milk which causes important changes in the price of these products. In northern Mexico for instance, 80% of females in extensive conditions give birth between November and February. Even if the price of milk is almost constant all the year around, the producers wish for kidding out of the normal season to reduce the seasonal fluctuations in their income, the main source of which is the milk sold. Moreover, the price of kids born in September and October are 30-50% higher than that of kids born during the natural kidding period. For these reasons, producers wish to manipulate the sexual activity of animals in order that they kid when the market conditions are optimal. In goat and sheep breeds from temperate latitudes, seasonality in females has been overridden using both exogenous hormones (progestagens and eCG) and photoperiodic treatments, while in males these latter treatments have induced sexual activity during the non-breeding season (Delgadillo et al., 1991; Chemineau et al., 1992; Leboeuf et al., 1998). However, these techniques are rather expensive and hard to

apply in flocks maintained in the extensive management conditions generally existing in subtropical and tropical regions. Under these circumstances, the techniques used to induce sexual activity during seasons of anoestrus (females) or rest (males) must be simple and non-expensive if they are to be integrated to the production systems of these latitudes. One such technique is the male effect (teasing) which allows induction of reproduction in seasonally anovulatory females by the introduction of a male. However, studies of the sexual characteristics of the animals, and identification of the environmental factors responsible for the annual period of sexual rest in both sexes, are necessary for the successful application of this technique.

Photoperiod, the major environmental factor responsible for reproductive seasonality in animals in subtropical Northern Mexico

As mentioned above, local goats of subtropical Mexico display an annual period of anoestrus when they are maintained in extensive conditions. This reproductive resting season coincides with the period of dry weather and scarcity of food. However, large seasonal variations in sexual activity are observed even in animals subjected to intensive conditions and fed a constant diet. This shows that food availability is not responsible for seasonal changes in sexual activity.

In females maintained in good body score and feeding conditions under natural daylength, the ovulatory activity starts on average on September 5 and finishes on February 23 (Duarte et al., 1999). In males raised under the same favourable conditions, sexual activity lasts from May to December (Delgadillo et al., 1999). The year to year repeatability in timing of reproductive activity in local male and female goats from subtropical Mexico suggests that a very reliable environmental factor, such as photoperiod, is used by these animals to synchronise their sexual activity. This was shown by subjecting females goats to alternating periods of 3 months of long days and 3 months of short days over 2 consecutive years. Under these conditions, ovulations ceased on average 19.2 ± 1.2 days (mean ± S.E.M.) following exposure to long days and started 67.4 ± 2.3 days following exposure to short days (Duarte et al., 1999). In males, alternating between 3 months of long days and 3 months of short days also disrupted the seasonality observed under natural conditions. Testicular weight started to increase in the middle of the period of exposure to long days and reached its maximum in the middle of the period of exposure to short days (Cortez-López, 1997).

These data demonstrate that local goats of Northern Mexico are responsive to the photoperiodic changes encountered at this latitude. These results are also very interesting because they suggest that it could be possible to induce sexual activity in males, and therefore use them as teasers to stimulate oestrus and ovulation in seasonally anoestrous females using the "male effect".

Male effect (teasing), a useful tool for inducing out-of-season reproductive activity in goats

The introduction of a male can induce sexual activity within a few days in anovulatory females that were previously separated from any contact with males. This phenomenon, known as the "male effect" or "teasing", has been extensively studied in ewes (Martin et al., 1986; Signoret, 1990) and goats (Chemineau, 1987; Walkden-Brown et al., 1993). Male contact induces a rapid increase in the frequency of plasma LH pulses culminating in a preovulatory LH surge and ovulation (Poindron et al., 1980; Martin et al., 1986; Chemineau, 1987). In goats, this induced ovulation is associated with oestrus in about 60% of does and is followed in 75% of cases by a 5-7-day short ovarian cycle. This short cycle is always followed by a second ovulation, which is associated with oestrus in 90% of females, and a normal luteal phase (Chemineau, 1987). A major limitation of the male effect for induction of reproductive activity in females is that it is weak when performed during seasonal anoestrus, especially in breeds of sheep or goats that are strongly seasonal. Thus, in breeds exhibiting only a moderate seasonality such as the Merino sheep or Creole goats of Guadeloupe Island, male introduction can induce an ovulatory response at any time throughout the anoestrous season (Lindsay and Signoret, 1980; Chemineau, 1983). However, in more seasonal breeds, the male effect is limited to about one month before the onset and one month

Figure 1

Sexual behavior observed for two hours daily in the first five days of teasing in sexually inactive males that had been maintained under natural variations of photoperiod (□ ; n = 4), and in sexually active bucks that had been treated with 2.5 months of long days and two implants of melatonin (■ ; n = 4). ***Differences between two groups (P < 0.001).

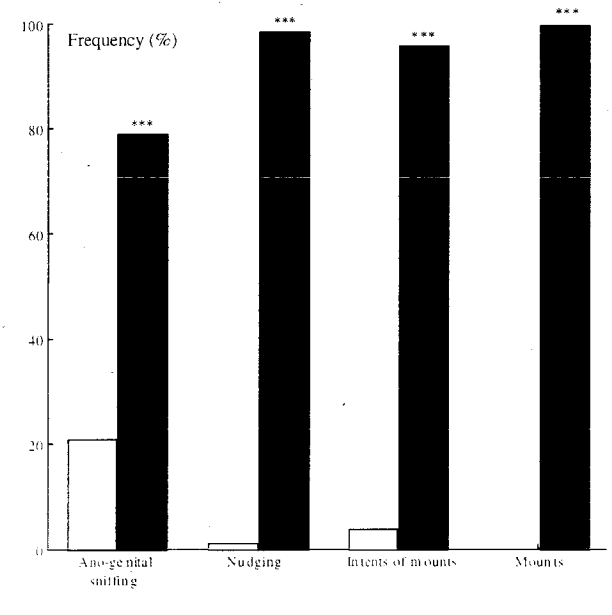


Figure 2

Sexual behavior observed for two hours daily in the first five days of teasing with does of experiment 2. The sexually inactive bucks had been maintained under natural variation of photoperiod (□ ; n = 4), while sexually active bucks had been previously treated with 2.5 months of long days and two implants of melatonin (■ ; n = 4). * Differences between SI and SA groups (P < 0.001).**

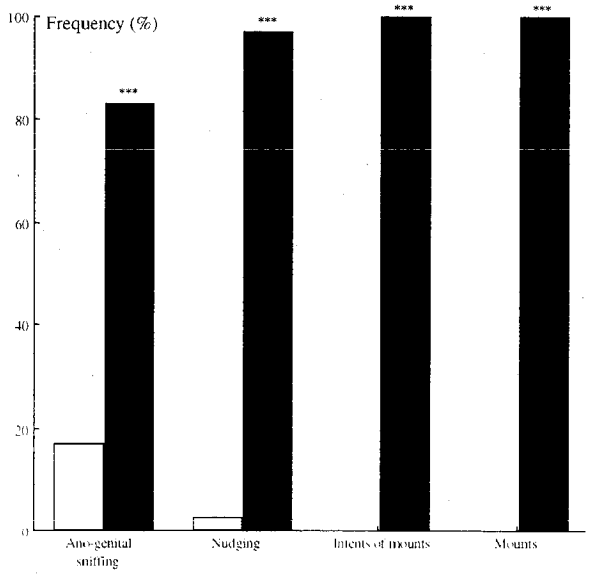


Figure 3
Proportion of females displaying oestrous activity after introduction of sexually active bucks which were treated with 2.5 months of long days followed by melatonin implants in a group of seasonally anovulatory females (solid circles; n = 40). Overall, no estrus was observed in a group of females teased with sexually inactive bucks during the same period (open circles; n = 34: P < 0.0001). Day 1 is day of teasing.

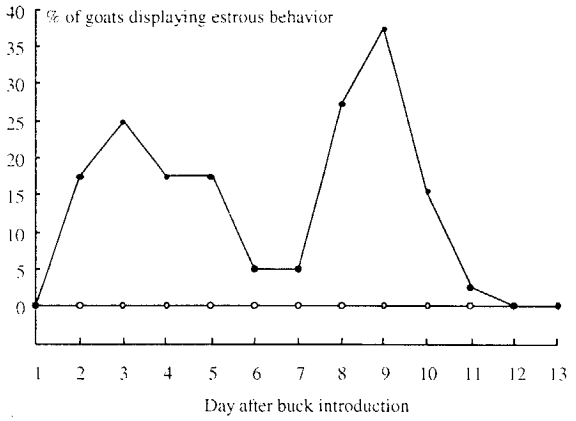
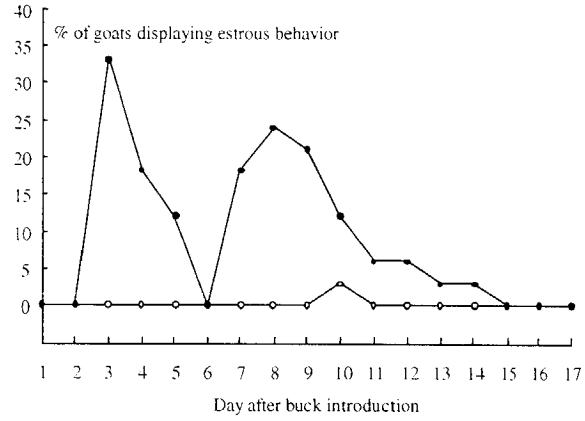


Figure 4.
Proportion of females displaying oestrous activity after introduction of sexually active bucks which were treated with 2.5 months of long days followed by melatonin implants in a group of seasonally anovulatory females (solid circles; n = 40). Overall, no estrus was observed in group of females from the same flock teased with sexually inactive bucks during the same period (open circles; n = 34: P < 0.0001). Day 1 is day of teasing.



after the end of the normal breeding season (Martin and Scaramuzzi, 1983; Chemineau, 1987). The failure of teasing outside this limited period may be due to the inability of the female to respond during anoestrus, resulting from refractoriness of the female to the male stimulus. Alternatively, it may be due to inadequate stimulation by the male. Indeed, male behavioural and physiological activity decreases during the period of female anoestrus in both sheep (Lincoln and Short, 1980) and goats (Delgadillo et al., 1991; 1999). It is well established in these species that physical contact or increasing the sexual activity of the males enhances the female response (Cohen-Tannoudji et al., 1986; Walkden-Brown et al., 1993). Also, the emission of olfactory cues from the male may be reduced during sexual rest.

Thus, it would be interesting to test the response of females goats to the male effect during seasonal anoestrus using bucks previously made sexually active by a treatment with long days followed by melatonin implants.

Response of males to photoperiodic treatment

In the first year, 4 males (Sexually inactive-SI; n = 4) perceived natural photoperiodic variations (13 h 41' of light at the summer solstice and 10 h 19' of light at the winter solstice) throughout the study. Another 4 males (Sexually active-SA; n = 4) were subjected to a treatment of long days (16 h of light/8 h of darkness) from November 1 to January 15 in open sheds. On January 16, each male received two subcutaneous implants each containing 18 mg melatonin (Regulin-Mélovine, SANOFI SNA, Libourne, France). At this time, the light treatment was stopped and the males were exposed to the natural day length until the end of the study. During the study, the animals were kept outdoors and fed alfalfa *ad libitum* and 300 g of commercial concentrate (14% proteins, Generaleche, Purina) with free access to mineral blocks and water.

The sexual behaviour of the bucks, observed for 2 h daily during the first 5 days after the males were introduced to the females, differed highly significantly between the two groups (Figure 1). The males treated with long days and melatonin displayed a high level of sexual activity, with genital sniffing, nudging, intents of mounting and mount frequencies all being much higher in the treated bucks than in controls (Figure 1; P < 0.001 for all comparisons). In the second year, treated males (n = 3) again displayed more sexual behaviour than controls (n = 3; P<0.001 in all cases; Figure 2).

Overall, these results show that, in Mexican local goats, a treatment of long days followed by melatonin induces an intense sexual behaviour during the period of sexual rest. Our data agree with those reported in male Ile-de-France and Suffolk sheep also treated with long days followed by insertion of subcutaneous melatonin implants (Hanif and Williams 1990, Chemineau et al., 1992). This intense sexual behavior out-of-season could be used to induce oestrus and ovulations in out-of-season female goats.

Response of Females to Male Effect

To study the response of female goats to the male effect, two experiments were performed during 2 consecutive years. In the first year, the experiment was carried out on two farms 4 km apart, to avoid any risk of interaction between the experimental and control groups. In the second year, the experiment was repeated using a single flock divided into two groups to test whether the difference in response between the control and the experimental group found the first year was due to some uncontrolled difference in the responsiveness of the two flocks.

In the first experimental year, the SI and SA males were introduced on March 15 into two groups of anovulatory females goats (SI n = 34; SA n = 40) belonging to 2 different flocks. They remained among the females for 35 days. Only 2 does in contact with SI males showed a silent ovulation between days 5 and 7 after male introduction. No further ovulatory or behavioural activity was observed during the remainder of the study. In contrast, all females exposed to SA males ovulated and showed at least one period of oestrous behaviour during the first 11 days following male introduction (P < 0.001). Thirty-two of 40 goats ovulated between day 1 and 6, of which 26 showed oestrous behaviour during this period. Twenty-eight of 32 females displayed a short ovarian cycle and ovulated a second time between days 7 and 11, while the other 4 does showed a normal ovarian cycle. Eight of 40 females ovulated between days 7 and 11. All the ovulations detected between days 7 and 11 were associated with oestrous behaviour. After day 12, no ovulations or oestrous behaviours were recorded (Figure 3). In does that showed an early response (days 1 to 6), the interval between the introduction of males and the onset of oestrous behaviour was 3.5 ± 0.2 days. In does which responded later (days 7 to 11), this interval was 9.1 ± 0.4 days. Overall, 38 of 40 females stimulated with treated bucks were diagnosed pregnant at 35 days, according to progesterone assay (versus 0 in the SI-treated group: P<0.001). The fertility at parturition was 60% with a prolificacy of 2.0 ± 0.1 in the SA group, whereas no kidding was observed during the same period in the SI group.

Similar results were obtained during the second experimental year. On March 15, the SI and SA males were introduced into two groups of anovulatory females belonging to the same

flock (n = 33 in each group). One doe in contact with SI males showed oestrus on day 10 after introducing the males. In contrast, 82% (27/33) of the does in contact with SA males showed oestrus between day 3 and day 14 after the beginning of teasing (Figure 4). The fertility at parturition was 64% with a prolificacy of 1.9 ± 0.1 in the SA group, whereas no kidding was observed during the same period in the SI group.

Our results clearly show that sexual activity can be induced in anoestrous females when they are exposed to males that have been treated with long days and melatonin and are thus showing a level of sexual behaviour similar to that of the natural breeding season. Because sexually active and inactive males were used, our results suggest that the absence of induction in the control groups is due to reduced stimulation by the male rather than to unresponsiveness of the female.

Of the females in contact with SI males, only 2 does ovulated and none showed oestrus throughout Experiment 1 and only one doe displayed oestrus in Experiment 2. This data is not in agreement with the results of Chemineau et al. (1987) who reported that the reintroduction of males in a group of goats stimulated their sexual activity in the following days. In contrast, in the females in contact with SA males, more than 80 % displayed a sexual activity during the first 14 days after the male introduction. In this latter case, the response of females to male effect was similar to that reported earlier in ewes and goats (Martin et al., 1986; Chemineau et al., 1987). These results were obtained in two consecutive years using anovulatory animals of different flocks (year 1) or of a single flock (year 2), so they clearly demonstrate that, at least under subtropical latitudes, the sexual behaviour of males is an important component in the response of does to the male effect. In future studies it would be useful to determine if the presence of oestrous females at the moment of male introduction can modify the response of females to the male effect, as it was suggested by Walkden-Brown et al. (1999).

CONCLUSION

The male effect is an effective method for inducing synchronous cyclic reproductive activity during seasonal anoestrus, but only if fully sexually active bucks are used. The failure of the male effect during anoestrus in goats, at least under subtropical latitudes, is not due to the existence of some total physiological or sensory refractoriness of the female to the stimulation by the male. Rather, it appears that the stimulus provided by the male at this time of the year is insufficient. It is generally accepted that teasing is best used at the beginning or at the end of seasonal anoestrus, and that it is unreliable at the time of deepest anoestrus. In the light of our present results, it would be interesting to investigate whether strongly seasonal ewes and goats would be able to respond to the presence of males previously treated with light and melatonin, or even with light alone.

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