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Presence of a sexually active goat buck enhances ovulation occurrence in seasonally anestrous does after ovulation and luteolysis induction in hormonally-treated goats in seasonal anestrus

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ABSTRACT

In seasonally anestrous goat does, ovulations can be induced by combining a treatment regimen including progestagen, eCG and prostaglandins. Nonetheless, ovulations occur only once and then does return to a seasonally anestrous state. This study was performed to determine whether the presence of a sexually active buck can stimulate a second ovulation after induced luteolysis using prostaglandins following the first ovulation. Three groups of seasonally anestrous does were treated to induce ovulations using an intra-vaginally inserted sponge containing a progestin combined with eCG and prostaglandin administrations. Goats that had ovulations were treated with a prostaglandin 11 days after progestin sponge removal. After the prostaglandin injection, does continued to be isolated from bucks (n = 8), were penned with a control buck (n = 9), or were penned with a sexually active buck (n = 10). The proportion of goats having ovulations after imposing the ovulation-induction protocol was greater than 80% and did not differ among treatment groups ($P >$ 0.05). The proportion of does having ovulations after injecting prostaglandins was greater when does were penned with a sexually active buck (8/10) than does penned with a control buck (0/9) or that were isolated from bucks (0/8; $P <$ 0.05). It is concluded that in seasonally anestrous goat does induced to have ovulations using a hormonal treatment regimen, the presence of a sexually active buck can induce a second ovulation when there is an induced luteolysis.

1. Introduction

Reproductive seasonality is a common characteristic in breeds of goats maintained at subtropical latitudes (Rivera et al., 2003;

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Lassoued and Rekik, 2005; Duarte et al., 2008). In these goats, photoperiod is the main environmental factor responsible for the reproductive seasonality, which modifies the estradiol negative feedback on LH secretion (Chemineau et al., 1988; Duarte et al., 2008, 2010). There is a marked increase in estradiol negative feedback on LH secretion during the seasonal anestrous period immediately after the last luteolysis that occurs during the breeding season (Legan et al., 1977), reducing the frequency of LH pulse secretion and thus preventing the occurrence of ovulations (Mori et al., 1987; Chemineau et al., 1988; Duarte et al., 2008). Hence, in goat does, variations of estradiol negative feedback on LH secretion is the primary neuroendocrine mechanism by which photoperiod regulates reproductive seasonality.

Reproductive seasonality is an important limitation for goat production systems in subtropical and at temperate latitudes. There, therefore, has been development of various treatments to modify this seasonality, including the imposing of treatments to result in the "male effect" and ovulation induction protocols (Leboeuf et al., 2003; Delgadillo et al., 2009). The placement of bucks in close proximity with a group of seasonally anestrous goat does stimulates ovulation to occur within the first 5 days after placement of the bucks with the does (Ott et al., 1980; Pellicer-Rubio et al., 2016). Interestingly, the placement of photo-stimulated sexually active bucks with does resulted in a greater number of does having ovulations than placement of does in close proximity with sexually inactive bucks (Bedos et al., 2014; Chasles et al., 2016; Zarazaga et al., 2019). Additionally, in intact or ovariectomized goat does treated with a subcutaneous estradiol implant, the placement of sexually active bucks in close proximity to does stimulated LH secretion in the does, whereas the placement with the control bucks did not affect LH secretion patterns (Vielma et al., 2009; Bedos et al., 2014; Muñoz et al., 2017). When results from all these studies are considered, there are indications that sexually active bucks induce a cessation of the negative feedback effect of estradiol on LH secretion during the seasonal anestrous period, thus, there is an increase in frequency of release of LH pulses leading to ovulation.

Ovulation induction protocols are also used to induce and synchronize the time of ovulations among does during the seasonal anestrous period. The most frequently used treatment includes progestagens, equine chorionic gonadotropin (eCG), and prostaglandin analogues (Leboeuf et al., 2003). Goat does generally have ovulations once after imposing this treatment regimen and then there is a return to the seasonally anestrous state (Corteel et al., 1988), thus, there is a minimal opportunity for getting does bred because of the lack subsequent estrous cycles. This return to the anestrous state is probably because of the marked negative feedback of estradiol on LH secretion after the last luteolysis of the breeding season (Legan et al., 1977; Chemineau et al., 1988; Muñoz et al., 2017). The presence of sexually active bucks with does has a marked effect on negating the negative feedback of estradiol on pulsatile LH secretion during seasonal anestrus, resulting in does continuing to have estrous cycles associated with ovulations after hormonal-based induction of ovulations in seasonally anestrous does. The working hypothesis for the present study, therefore, was that in goat does induced to have ovulations and development of corpora lutea using a hormonal protocol during seasonal anestrus, the presence of a photo-stimulated buck would result in ovulations after induction of luteolysis of the induced corpora lutea. To test this possibility, three groups of does were studied: does continued to be isolated from bucks (n = 8), were penned with a control buck (n = 9), or were penned with a sexually active buck (n = 10).

2. Materials and methods

All procedures were performed in accordance with the Official Mexican Rule for the technical specifications for the production, care, and use of laboratory animals (SAGARPA, 2001).

2.1. General conditions of the study

This study was conducted in the Laguna region (latitude 26° 23´N, longitude 104° 47´W), State of Coahuila, in northern Mexico. In the Laguna region, the natural photoperiod varies from 13 h 41 min of light at the summer solstice to 10 h 19 min of light at the winter solstice. Female goats maintained in the Laguna region that are isolated from males are seasonally anestrus from February to September (Duarte et al., 2008). Bucks isolated from females have less, as compared to what occurs during the breeding season, testicular hormone production, gamete production, and sexual behavior from December to June (Delgadillo et al., 1999). In the present study, there was housing of bucks and does in open shaded pens during the entire study. Each animal was fed 2 kg of alfalfa hay (18% CP) and 200 g of commercial concentrate feed (14% CP; 1.7 Mcal/kg). Water and mineral blocks were provided *ad libitum.*

2.2. Photoperiodic treatment to stimulate the sexual activity of bucks

There was use of 4-year-old vasectomized bucks that had previous sexual experience. There was induction of sexual functions in one buck during the non-breeding season by imposing a photo-stimulatory treatment. This buck was housed in a shaded open pen with natural photoperiodic conditions, and there was imposing for 2.5 months of a long-day photoperiod, using artificial light in the morning (06:00 to 08:00) and at night (18:00 to 22:00) so that the total photoperiod was 16 h of light per day from 1 November to 15 January (Delgadillo et al., 2002). This buck was considered to be the photo-stimulated, sexually active buck. There was housing of another buck in a shaded open pen with natural photoperiodic conditions, thus, there was no photoperiodic sexual stimulation of this buck during the entire period the study was conducted. This buck was considered to be the control buck.

2.3. Hormonal treatment to induce ovulations during seasonal anestrous

There were 3 to 5-year old multiparous goat does used to conduct this study. In March, does were subjected to three consecutive trans-rectal ultra-sonographies with 7 or 8 days between assessment periods using an Aloka SSD-500 machine connected to a trans-rectal 7.5 MHz linear probe to determine the doe ovulatory status. The absence of the corpus luteum at the time of the three ultra-sonographies was considered to indicate does were seasonally anestrus (Delgadillo et al., 2011). None of the does had a corpus luteum, therefore, all does were considered to be seasonally anestrous. On 27 March (Day 0), does were allocated to three groups ($n = 10$ per group) balanced for BCS. Immediately after treatment allocations, all does were treated with a hormonal protocol to stimulate ovulations (thereafter referred to as “induction protocol”) during seasonal anestrus with the treatment regimen used being based on results from a previous study of Leboeuf et al. (2003). An intravaginal sponge containing 20 mg of cronolone (Chronogest CR, Intervet laboratories, Estado de Mexico, Mexico) was placed in the vagina and remained in place for 12 days. On Day 10, each female was treated im with 300 IU of eCG (Folligón 500 IU/mL, Intervet laboratories, Estado de Mexico, Mexico), and 125 μg of synthetic prostaglandins (Ciclofer 175 μg/mL; Cifer laboratories, Durango, Mexico). This hormonal treatment induces release of a LH surge and ovulation occurs in most does between 24 and 42 h after sponge removal (Leboeuf et al., 2003).

2.4. Hormonal treatment to induce luteolysis and contact with bucks

On 7 April (i.e., 11 days after sponge removal) each doe that had at least one ovulation after imposing the ovulation induction protocol was treated im with 125 μg of synthetic prostaglandins to induce luteolysis. Immediately after administration of the prostaglandin, does continued to be isolated from bucks throughout the study ($n = 8$; BCS: 2.1 ± 0.1), were penned with a control buck ($n = 9$; BCS: 2.2 ± 0.1); or were penned with a sexually active buck ($n = 10$; BCS: 2.2 ± 0.1) The distance between the three groups was at least 200 m to avoid any inter-group treatment effects.

2.5. Variables assessed

Ovulation after the imposing ot the induction protocol was determined by the presence of corpora lutea in each female using trans-rectal ultra-sonography performed 11 days after sponge removal (7.5-MHz, Aloka SSD-500 device; Delgadillo et al., 2011). Occurrence of a second ovulation after prostaglandin administration was determined by quantifying plasma progesterone concentrations. For this purpose, there was collection of 5 mL daily blood samples from each female from the day of prostaglandin administration until day 6 after administration. In addition, blood samples were collected 9, 12 and 15 days after treatment with the prostaglandin. All blood samples were collected using jugular venipuncture procedures into tubes containing 30 μL of heparin. The samples were centrifuged at 3000 x *g* for 30 min, and the obtained plasma was stored at -20 °C until progesterone concentrations were determined using the immuno-enzymatic technique described by Canépa et al. (2008). The sensitivity of the assay was 0.25 ng/mL, and the intra-assay CV was 8%. Ovulations were considered to have occurred when progesterone concentrations were ≥1.0 ng/mL (Chemineau et al., 2006).

2.6. Statistical analyses

The proportion of does having ovulations after the induction treatment or after the prostaglandin injection and placement of bucks with does were analyzed using the Chi-square test. When there was a statistical difference, the comparisons among groups were made using the Fisher's exact test. Differences among groups were considered significant at $P \leq 0.05$. Statistical analyses were performed using the statistical package SYSTAT 13 (2009).

3. Results

3.1. Ovulatory response to the presence of bucks

More than 80% of the does had ovulations in response to the induction protocol, and these proportions did not differ among groups ($P > 0.05$; Table 1). By contrast, the proportion of does that had ovulations after the administration of prostaglandins differed among the three groups of does ($P < 0.01$). This proportion was greater in does penned with a sexually active buck than in those penned with the control buck or isolated from bucks ($P < 0.05$; Table 1). The individual profiles of plasma progesterone concentrations indicated that about 9 days after prostaglandin administration, progesterone concentrations increased in does penned

Table 1
Response of goat does induced to have ovulations during seasonal anestrous period using an ovulation-induction protocol. Does that had ovulations were treated with an injection of prostaglandin 11 days after the end of the treatment regimen. Immediately after treatment with prostaglandin, does continued to be isolated from bucks; were penned with a control sexually inactive buck; or were penned with a vasectomized buck that was sexually active due to a photo-stimulatory treatment.

Groups	*n*	Does having ovulations after hormonal treatment	Does having ovulations after treated with prostaglandins
Isolated goats	10	8/10[a]	0/8[a]
Penned with control buck	10	9/10[a]	0/9[a]
Penned with sexually active buck	10	10/10[a]	8/10[b]

[a,b] Different superscripts within each column indicate a difference ($P < 0.05$).

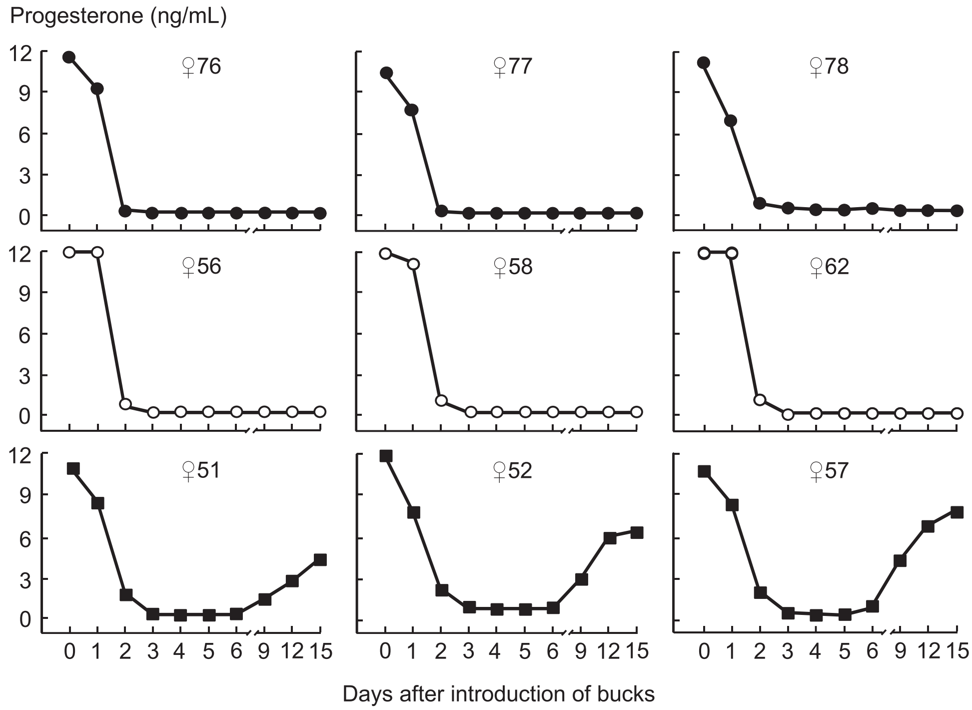

Fig. 1. Individual profiles of plasma progesterone concentrations in goat does induced to have ovulations during the seasonal anestrous using an ovulation induction protocol. Does that had ovulations were treated with an injection of prostaglandin 11 days after completion of the treatment regimen. Immediately after treatment with prostaglandin, does continued to be isolated from bucks (●), were penned with a control buck (○), or were penned with a sexually active buck (■).

with a sexually active buck, indicating ovulation had occurred. By contrast, progesterone concentrations remained basal in goats penned with the control buck or in those isolated from bucks, indicating these does were in an anestrous state (Fig. 1).

4. Discussion

Results from the present study support the working hypothesis that in goat does induced to have ovulations during seasonal anestrus using a hormonal treatment regimen, the presence of a sexually active buck prolongs the cyclical pattern of ovulations when luteolysis is induced after the first ovulation by administration of prostaglandins. In contrast, none of the does penned with the control buck or isolated from the buck had ovulations after the prostaglandins treatment. These findings are consistent with previous results where the placement of sexually active bucks with does induced ovulations in seasonally anestrous does, whereas control bucks expressing little sexual behavior did not induce ovulations in seasonally anestrous does (Delgadillo et al., 2015).

The presence of control bucks with does or when there is doe isolation from bucks, there is not a continuation of the cyclic pattern of ovulations that prevails during the breeding season. These findings are consistent with results in previous studies where seasonally anestrous does that were induced to have ovulations had a return to the anestrous state after ovulation induction (Corteel et al., 1988; Leboeuf et al., 2003). The fact that does have ovulations only once after imposing the hormonal protocol for ovulation induction limits the opportunity for getting does bred because this protocol is generally used when artificial insemination (AI) is being used for breeding. If does are not pregnant after the AI, there is no second opportunity for does to become pregnant because there is a return to the seasonally anestrous state. The main outcome in the present study is that the presence of a sexually active buck prolongs the ovarian functions of most does after an induced luteolysis. This outcome could have important practical applications. Non-pregnant does could be inseminated a second time at a naturally occurring estrus if vasectomized-sexually active bucks are used for sexual stimulation of does. Alternatively, entire (intact) sexually active bucks could be used to mate females that did not become pregnant as a result of AI. The presence of sexually active bucks results in a continuance of estrous cyclic patterns in most females after an induced (the present study) or naturally occurring luteolysis (Delgadillo et al., 2015). The use of sexually active bucks in a management regimen could lead to an increase in the fertility in goat herds. When all the results from the present study are considered, there are indications that there can be use of sexually active bucks to improve the management strategies of reproduction in seasonally anestrous goats.

It is well established that the continued presence of sexually active bucks is an important factor in stimulation of a greater frequency of pulsatile LH secretions and, therefore, induction of ovulation during the period of seasonal anestrus in multiparous or pre-pubertal does (Vielma et al., 2009; Martínez-Alfaro et al., 2014; Chasles et al., 2018, 2019). During seasonal anestrus, the estradiol negative feedback is relatively greater as compared with what occurs during the breeding season, thus, there is a lesser frequency of LH pulse release and resulting lack of ovulation occurrence (Mori et al., 1987; Chemineau et al., 1988). The sudden introduction of sexually active bucks, however, induces an increase in frequency of LH pulse secretions or ovulation in intact or ovariectomized does treated with a subcutaneous estradiol implant, whereas with placement of control bucks with does there is not an induction of ovulations (Vielma et al., 2009; Bedos et al., 2014; Muñoz et al., 2017). In addition, with ovariectomized does, the presence of sexually active bucks prevents the seasonal decrease of LH that occurs with the onset of the anestrous season (Muñoz et al., 2017). The most likely explanation of the results in the present study is that the presence of the sexually active buck induced and increase in frequency of pulsatile LH release because of a decrease in the negative feedback of estradiol on LH secretion, maintaining the occurrence of the cyclic pattern of ovulations in does after the induced luteolysis.

5. Conclusions

In conclusion, in seasonal anestrous goat does induced to have an ovulation using a hormonal treatment regimen, the presence of a sexually active buck prolongs the cyclic pattern of ovulations after an induced luteolysis. The continuance of this cyclic ovulatory pattern allows for the opportunity to increase the proportion of pregnant does, thus improving the possibility of breeding during periods when does are typically seasonal anestrus.

Declaration of Competing Interest

The authors declare that there are no conflicts of interest.

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