

Male contact can replace equine chorionic gonadotropin (eCG) injection in anestrus progesterone-treated dairy goats

El contacto con machos puede reemplazar la inyección de gonadotropina coriónica equina (eCG) en cabras lecheras en anestro tratadas con progesterona

Contato com machos pode substituir injeção de gonadotrofina coriônica equina (eCG) em cabras leiteiras em anestro tratadas com progesterona

Débora Gamboa Beltrán₁
Lorenzo Álvarez Ramírez₂

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¹ Médica Veterinaria Zootecnista. Universidad Nacional Autónoma de México.

² Facultad de Medicina Veterinaria y Zootecnia, Universidad Nacional Autónoma de México.

E-mail: alorenzo@unam.mx

ORCID: <https://orcid.org/0000-0003-1019-2635>



Abstract

Introduction: Equine chorionic gonadotropin (eCG) is a crucial component of progestogen treatments to induce reproductive activity in goats. However, its repeated use leads to antibody production, and its origin raises animal welfare concerns. **Methodology:** This study compared the reproductive performance of anestrus dairy goats treated with eCG or exposed to males following a 14-day progesterone treatment. Seventy-one anestrus goats were implanted with a controlled internal drug release (CIDR®) device and placed in one of three groups: the eCG group (n = 23) received an injection of 200 IU of eCG, the male group (M, n = 24) was exposed to a male in the same pen for a period of seven days, and the control group (C, n = 24) received no additional treatment. **Results:** Estrus, ovulation, and pregnancy rates were recorded. Estrus rates and the interval to estrus (h±SE) in the eCG (95.7% and 36.4±2.8, respectively) and M groups (95.9%, 48.2 ± 2.7) were significantly different from those in the control group (58.3%, 62.7 ± 3.6; $p < 0.05$). Ovulation rates were higher in the eCG (91.3%) and M groups (83.3%) than in the C group (29.1%; $p < 0.05$). The pregnancy rate was higher in the eCG (91.3%) and M groups (79.1%) than in the C group (29.1%; $p < 0.01$). **Conclusion:** The results indicate that the male effect can replace eCG in progesterone-treated anestrus goats.

Keywords: biostimulation; estrus induction; sexual stimulation; social stimulation.

Resumen

Introducción: La gonadotropina coriónica equina (eCG) es un componente crucial de los tratamientos con progestágenos para inducir la actividad reproductiva en cabras. Sin embargo, su uso repetido conduce a la producción de anticuerpos y su origen plantea preocupaciones sobre el bienestar animal. Este estudio comparó el rendimiento reproductivo de cabras lecheras en anestro tratadas con eCG o expuestas a machos después de un tratamiento de progesterona de 14 días. **Metodología:** Setenta y una cabras en anestro fueron implantadas con un dispositivo de liberación interna controlada (CIDR®) y se colocaron en uno de tres grupos: el grupo eCG (n = 23) recibió una inyección de 200 UI de eCG, el grupo macho (M, n = 24) fue expuesto a un macho en el mismo corral durante un período de 7 días y el grupo control (C, n = 24) no recibió tratamiento adicional. **Resultados:** Se registraron las tasas de estro, ovulación y embarazo. Las tasas de estro y el intervalo hasta el estro (h±SE) en los grupos eCG (95.7% y 36.4±2.8, respectivamente) y M (95.9%, 48.2 ± 2.7) fueron significativamente diferentes de las del grupo control (58.3%, 62.7 ± 3.6; $p < 0.05$). Las tasas de ovulación fueron más altas en los grupos eCG (91.3%) y M (83.3%) que en el grupo C (29.1%; $p < 0.05$). La tasa de embarazo fue mayor en los grupos eCG (91.3%) y M (79.1%) que en el grupo C (29.1%; $p < 0.01$). **Conclusión:** Los resultados indican que el efecto macho puede reemplazar a la eCG en cabras en anestro tratadas con progesterona.

Palabras clave: biostimulación; inducción de estro; estimulación sexual; estimulación social.

Resumo

A gonadotrofina coriônica equina (eCG) é um componente crucial dos tratamentos com progestogênio para induzir a atividade reprodutiva em caprinos. No entanto, seu uso repetido leva à produção de anticorpos e sua origem trás preocupações sobre o bem-estar animal. **Metodologia:** Este estudo comparou o desempenho reprodutivo de cabras leiteiras em anestro tratadas com eCG ou expostas a machos após um tratamento de 14 dias com progesterona.

Resultados: A gonadotrofina coriônica equina (eCG) é um componente crucial dos tratamentos com progestogênio para induzir a atividade reprodutiva em caprinos. No entanto, seu uso repetido aumenta a produção de anticorpos e sua origem trás preocupações sobre bebidas de origem animal. **Conclusão:** Este estudo compara o desempenho reprodutivo de cabras leiteiras em anestro tratadas com eCG ou expostas a machos após 14 dias de tratamento com progesterona.

Palavras chaves: bioestimulação; indução do estro; estimulação sexual; estimulação social.

Introduction

Domestic goats are seasonal breeders. This seasonality is determined by the photoperiod, and reproductive activity begins as day length decreases [1]. The resulting reproductive seasonality is an adaptive measure that allows animals to be born at a time when environmental conditions favour their viability. Although it represents an advantageous genetic trait under natural conditions, from a production perspective, it is an obstacle that prevents kidding outside the natural season and leads to inconsistent milk availability throughout the year. This leads to seasonal production, which prevents the producer from producing substantial amounts of milk and its derivatives during periods of increased market demand [1–3].

Several strategies have been developed to induce and synchronize reproductive activity. A well-known method involves the use of progesterone or progestogens. The most used method to administer these hormones is a vaginal device such as the controlled internal drug release (CIDR®, Pfizer® Animal Health) [1,3–5]. These treatments are followed by an injection of equine chorionic gonadotropin (eCG) to improve follicular development, estrus, and ovulatory response [1]. Seasonally anestrous ewes fail to respond to progesterone-progestogen treatments if no gonadotropin is administered [6], and eCG improves estrus and ovulatory responses compared with a control condition [7]. During the breeding season, the absence of eCG in progesterone-treated goats causes a decrease in the number of ovulating animals [8], and injecting eCG increases estrus and pregnancy rates at the beginning of the breeding season [9]. In anestrous cows [10] and mares [11], the administration of eCG after progesterone treatment improves the ovulatory response.

Equine chorionic gonadotropin is a placental glycoprotein with simultaneous FSH-LH-like activity and is extracted from the serum of pregnant mares [12]; thus, its method of production has raised several animal welfare concerns [13]. Furthermore, the injection of eCG induces the production of anti-eCG antibodies that interfere with the effectiveness of subsequent treatments and have been directly associated with adverse effects on ovulation, pregnancy, and kidding rates [14–19]. Therefore, alternatives for use in animal production are needed [20].

Introducing a male into a group of goats can stimulate reproductive activity; this phenomenon is known as the male effect. The introduction of males leads to an acute increase in the female pulsatile LH secretion frequency, providing a significant gonadal stimulus for ovulation [1,21–24].

It is hypothesized that in progesterone-based treatments, the role of eCG can be replaced by exposure to a male at the end of the treatment. Despite the economic importance of the subject for profitable goat production, no studies have compared

the use of eCG with exposure to a male in anestrus goats after progesterone treatment. The aim of this study was to investigate whether introducing a male goat after progesterone treatment can elicit the same estrus and ovulatory responses in female dairy goats as eCG administration during the anestrus season.

Materials and methods

Location

The study was conducted between March and April on a commercial farm of the Caprinocultores Unidos de Guanajuato, located in Guanajuato, Mexico (20°33'00.9"N 100°41'39.7"W).

Animals and treatments

Seventy-one dairy goats (French Alpine, Saanen, Toggenburg; $>52 \pm 4$ kg bw) underwent a 14-day progesterone treatment with the CIDR® vaginal device. Upon removal of the vaginal implant (Day 0), goats were randomly assigned to one of three treatment groups: the eCG group (n=23) received an intramuscular injection of 200 IU of equine chorionic gonadotropin, the M group (n=24) was exposed to a sexually active adult male, and the control group (C, n=24) received no further treatment. Groups were in separate pens >20 m apart, with solid fences between them. Animals were fed alfalfa hay, maize silage, and concentrate three times a day (06:00, 12:00, and 19:00) and were automatically milked twice a day (06:30 and 16:00). The goats were in late lactation and had an average daily milk production of 1.9 ± 0.3 L.

The male goats were older than 2.5 years and had previous mating experience in the farm, with acceptable breeding records. For a period of two weeks prior to their use, they were exposed to the continuous presence of oestrogenised females to induce sexual activation [25,26]. For at least five months prior to the beginning of the study, males were housed in separate pens at more than 40 m from the females. They were provided with a diet of alfalfa hay and commercial concentrate.

Measurements and sampling

To verify anestrus conditions, progesterone levels were measured in all goats by collecting three blood samples every five days before CIDR® insertion. On Day 3, a second phase of sampling to measure progesterone levels was conducted daily until

Day 13 to assess the ovulatory response. During the following 10 days, sampling was performed every other day. The day of ovulation was determined by subtracting 2 from the day that progesterone levels surpassed 1 ng/ml and continued for three consecutive samples [27]. The samples were centrifuged, and the plasma obtained was frozen until analysis using a solid-phase radioimmunoassay (DPC, Diagnostic Products Corporation, Los Angeles CA, US). The assay sensitivity and intra-assay coefficient of variation were 0.02 ng/ml and 4.1%, respectively.

Twenty hours after CIDR® removal, a programme for recording estrus was initiated and performed twice a day over a week. Aproned males were used in sessions with a maximum of 5 min. A female was recorded as in estrus if she remained still while courted by the male and allowed the male to mount. If a female was recorded as in estrus in the eCG and C groups, she was mated with a selected buck. In the M group, a male with a marking harness was introduced after progesterone treatment and remained in the pen for seven days, allowing mating with all receptive females. Every day, the colour of the marker was changed to identify new females in estrus.

The pregnancy rate was determined by transabdominal ultrasound conducted on Day 65 after CIDR® removal.

Data analysis

Estrus, ovulation, and pregnancy rates as well as estrus and ovulation intervals were compared among groups. Descriptive statistics, analyses of variance (PROC GLM), and Fisher's exact test (PROC FREQ) were used [28].

Results

None of the animals showed progesterone levels indicative of ovulation before treatment initiation.

The percentages of goats in estrus were not different between the eCG and M groups, and both percentages were higher than that in the C group (95.7, 95.9 and 58.3%, respectively; $p < 0.05$; Table 1). The interval to estrus was different among the groups ($p < 0.05$, Table 1).

Table 1. Estrus, ovulation, and pregnancy rates in anestrus goats treated with progesterone and one of the following: a dose of equine chorionic gonadotropin (eCG), exposure to a male goat (male), or no additional treatment (control).

	eCG (n = 23)	Male (n = 24)	Control (n = 24)
Estrus rate (%)	95.7 ^a	95.9 ^a	58.3 ^b
Interval to estrus (h ± SE)	36.4 ± 2.8 ^a	48.2 ± 2.7 ^b	62.7 ± 3.6 ^c
Ovulation rate (%)	91.3 ^a	83.3 ^a	29.1 ^b
Interval to ovulation (days ± SE)	3.3 ± 0.1 ^a	4.4 ± 0.2 ^b	4.1 ± 0.2 ^b
Pregnancy rate (%)	91.3 ^a	79.1 ^a	29.1 ^b

Note. ^{a,b} *P* < 0.05 between treatments. All goats were previously implanted with the CIDR[®] device for 14 days.

Source: own elaboration.

Goats in the eCG group responded first, followed by those in the M group and finally those in the control group. Figure 1A shows the cumulative distribution of goats in estrus after CIDR[®] withdrawal.

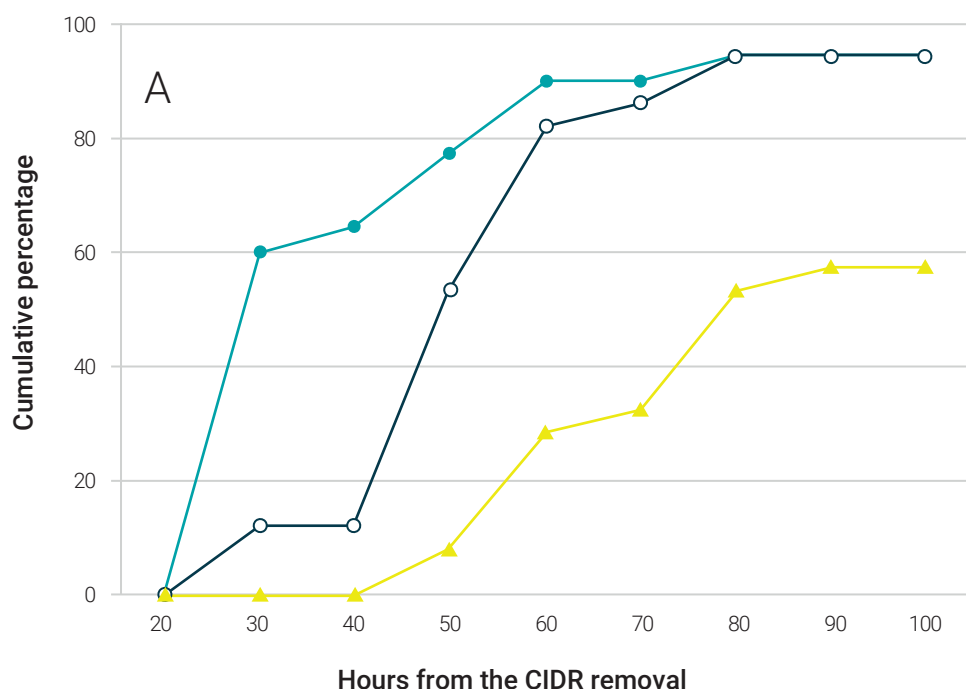
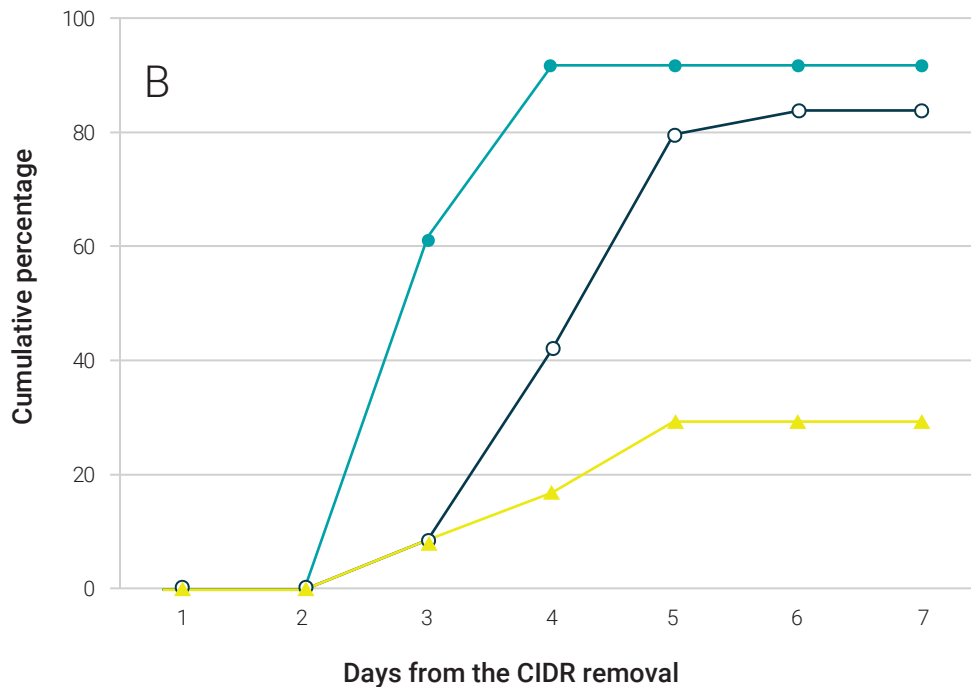


Figure 1. Cumulative distribution of estrus (A) and ovulation (B) in goats after CIDR removal. Anestrus goats were treated with progesterone and one of the following: a dose of equine chorionic gonadotropin (eCG, ●), exposure to a male (male, ○), or no additional treatment (control, ▲).

A lower ovulation rate was observed in the control group ($p < 0.05$). No significant differences in the ovulation rate were observed between the eCG and M groups ($p > 0.05$; Table 1).

Ovulation occurred at a shorter interval in the eCG group ($p < 0.05$), and no differences in the interval to ovulation were observed between the M and C groups ($p > 0.05$; Table 1). The cumulative distribution of ovulating goats is depicted in Figure 1B.



The pregnancy rate was higher in the eCG and M groups than in the C group ($p < 0.05$). No differences in the pregnancy rate were found between the eCG and M groups ($p > 0.05$; Table 1).

Discussion

The effectiveness of progestogen treatment during anestrus depends on adequate gonadotrophic stimulation [29]. The use of eCG for this purpose has serious drawbacks [13,16]. The present study showed that during the anestrus season, the reproductive response of anestrus goats treated with progesterone was maintained when eCG was replaced by contact with a male goat.

Taken together, the results of the present study support the hypothesis that introducing a male goat immediately after removal of the CIDR® leads to the same estrus and ovulatory responses as administering a dose of eCG. After using the CIDR® device, animals that received a dose of eCG or male contact exhibited similar estrus and ovulatory responses; these responses were superior to those of untreated goats. This result strongly indicates that the eCG and M groups experienced adequate gonadal stimulation after removal of the implanted vaginal device.

The male effect is characterized by an acute increase in gonadotropin secretion; LH secretion frequency and amplitude rise significantly [30], which may provide the gonadotrophic stimulation necessary for progesterone-treated goats to respond without requiring eCG injection. This promotes follicular development and increases oestradiol production, leading to estrus behaviour and ovulation [21], similar to the effects of administering eCG in hormonal treatments.

In this study, the M group exhibited estrus and ovulatory responses and pregnancy rates similar to those of goats receiving the conventional hormonal progesterone and eCG treatments [12,29,31]. Contact with male goats may have provided enough gonadal stimulation to induce follicular development, estrus, and successful ovulation in progesterone-treated goats that did not receive gonadotropin during seasonal anestrus. Interestingly, during the breeding season, a female effect has also been demonstrated to hasten estrus onset in progesterone-treated goats [32]. In our case, an acceptable reproductive response was not observed in progesterone-treated goats unless they were injected with eCG or had contact with a male after CIDR treatment during the anestrus season.

Positive results regarding the male effect have been reported in other studies in progesterone-treated goats. In a study conducted by Pellicer et al. [23] between April and June, goats exhibited high rates of estrus (> 90%), ovulation (> 80%), and pregnancy (78%) after 11 days of progesterone or progestogen treatment followed by 5 days of male contact; however, females in the aforementioned study underwent three months of photoperiodic treatment prior to the use of progesterone, whereas we found that this long treatment was not needed. On the other hand, Umberger et al. [6] demonstrated that a 10-day progestogen treatment alone is not able to induce a synchronized estrus response in seasonally anestrus ewes; when the progestogen treatment was followed by an eCG injection, the response was significant and similar to that when ram contact was allowed.

During the breeding period in Mexico, Mellado et al. [33] found that goats treated with Syncromate-B and eCG for 9 days showed a high estrus response when teased with a male, although they did not test other combinations of progestogen treatment

and male to determine whether male contact could replace eCG administration in estrus or anestrus goats. In estrus sheep, Nakafeero et al. [34] demonstrated that the ram effect could replace the administration of eCG in ewes receiving 12 to 14 days of progesterone treatment. Others have also demonstrated that the ram effect advances estrus and improves fertility following different prostaglandin synchronizing treatments during the breeding season [35,36]. In our study, we showed that the male effect successfully replaced eCG administration in progesterone-treated goats during seasonal anestrus.

Social cues can strongly affect female goat reproduction. The presence of sexually active males [21,30] or females [37,38] can induce estrus and ovulation. This phenomenon can reduce the dependence of hormonal treatments and their negative effects in the animal industry; its inclusion in the regular management of animals would help create “green, clean and ethical” management systems [39]. Overall, our findings support the effectiveness of male contact as a viable alternative solution to reduce the use of traditional hormonal therapies in goat breeding and to potentially reduce the cost of classic hormone therapies. In addition, replacing eCG administration with male contact can prevent the formation of anti-eCG antibodies and the association fertility reduction reported in subsequent treatments [14,19,40], which represents one of the major challenges of using hormonal estrus control [1]. When used in this manner, male contact might significantly reduce the use of hormonal treatments while ensuring optimal results.

In conclusion, male contact induced estrus, ovulatory, and pregnancy responses similar to those elicited by eCG injection in progesterone-treated anestrus goats.

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Conflict of interest

The authors declare that there are no conflicts of interest.

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