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Evaluation of Different eCG Doses + Progesterone to Induce Reproductive Activity During the Transitional Reproductive Season in Anestrous Creole Goats

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ABSTRACT

Background: Goats from subtropical and temperate latitudes show reproductive seasonality. For this reason, the products obtained from goats also shows the same seasonality, reducing the finances of goat keepers. Several studies have focused on reducing reproductive seasonality through the use of hormonal protocols based on progestogens, in addition to the use of equine chorionic gonadotropin (eCG) hormone, used for its double biological activity that ensures an optimal response to induce estrus and pregnancy in goats. The objective was to assess whether reduced doses of eCG are effective in inducing reproductive activity in anestrous goats.

Methods: During the transition reproductive period (June), mix-breed Creole goats (n=39), were treated with intramuscular progesterone (P4), later, the experimental treatments, consisting in different doses (50, 100, 50+50 or 200 IU) of equine chorionic gonadotropin (eCG) were applied. After the application of the experimental protocols, the reproductive activity of the goats was evaluated.

Result: This study shows that one dose of 100 IU of eCG effectively induced reproductive behavior in anestrous Creole goats towards the end of the seasonal anestrus. This protocol makes the use of exogenous hormones more efficient, with reduce doses, decreasing expenses and is practical use by goat producers.

Key words: Anestrus, eCG doses, Estrus and ovulation induction, Goats, Progesterone priming.

INTRODUCTION

Goats from subtropical and temperate latitudes are characterized for showing a marked reproductive seasonality (Duarte *et al*., 2008). For this reason, the supply of products obtained from goats (milk and kid meat) also show the same seasonality, reducing in parallel a continuous economic support by the goat keepers (Delgadillo, 2011). Several studies have centered their attention to decrease such reproductive seasonality through the use of hormonal protocols based on the use progestogens, mainly fluorogestone (FGA; 20-40 mg/sponge) and medroxyprogesterone acetate (MAP; 60 mg/sponge). Besides, controlled internal drug release (CIDR) devices are also used, normally impregnated with 0.30 g of natural progesterone (P_4), (Abecia *et al.,* 2012). The use of these intravaginal devices has been reduced to 5 d (Martemucci and D'Alessandro, 2011). In addition, protocols based on equine chorionic gonadotropin (eCG) have been used and displayed a dual biological activity; its primary action is similar to that of the follicle stimulating hormone (FSH), but it has a second action as luteinizing hormone (LH), (Simões, 2015). The afore mentioned actions assure an optimum response to induce estrus and pregnancy in goats (Simões, 2015; Rahman *et al.,* 2017). In goats, the interval between doses of eCG ranges from 200 to 1000 IU, depending on breed, age and season of the year (Najmi *et al.,* 2011; Abecia *et al.,* 2012). Nonetheless, these hormonal protocols have a major drawback, such as vaginal wall imparment, need for skilled labor, adequate facilities and equipment (Manes *et al*., 2015). When considering both public health, animal wellbeing, and productive sustainability, the use of high doses of exogenous hormones and prolonged administrations are being questioned (Simões, 2015). On this respect, Contreras-Villarreal *et al*. (2016), evaluated the use of a sole dose of 25 mg i.m. progesterone plus 250 IU of eCG 24 h apart, which induces 100% of estrus and

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ovulation in goats during the seasonal anestrus. Besides, Alvarado-Espino *et al.* (2016) achieved a reduction of the dose of human chorionic gonadotropin (hCG) down to 50 IU, in the induction of reproductive activity in anovulatory goats. This hormonal protocol also considered the use of 20 mg i.m. of progesterone, administered 24 h prior to hCG. For such evidences, the objective of the present study was to assess whether reduced doses of eCG are effective ininducing reproductive activity in anestrous goats.

MATERIALS AND METHODS

Ethics statement

All experimental procedures were compliant with the guidelines for ethical use, care and welfare of animals in research at international (FASS, 2010) and national (NAM,2002) levels with institutional approval reference number UAAAN-UL/02-03-1402-2620.

Experimental animals and their management

This research took place in the in Ejido Eureka, Gomez Palacio, Durango, México (25°49' North, 03°23' West) during June 2017, month that corresponds to the transition period from seasonal anestrus to reproductive activity, in goats under an extensive system. Mix-breed Creole goats (n=39) homogeneous by body weight 36.94 ± 7.53 kg and body condition score 2.18 ± 0.29 (Walkden-Brown *et al*., 1997; 1 to 4 scale) were randomly distributed in 35 m^2 pens. Feeding was based on alfalfa hay, water and salts *ad libitum.* In addition to 200 g of a commercial concentrated per goat/ day (14% CP). To define anestrus status of goats, two transrectal ultrasounds were performed (Fig. 1) by an experienced operator, using an echograph (Aloka 500) provided with a human prostate transducer of 7.5 MHz (Mhz linear array; Corometrics Medical Systems, Inc., Wallingford, CT, USA). At the beginning of the scanning, the goats were placed in vertical position and the transducer was covered with carboxymethylcellulose as a lubricant. Once the transducer was inserted in the rectum, both ovaries were scanned to determine the presence and type of ovarian structures. The anovulation in the goat was determined by absence of corpora lutea in the two ovaries observed.

Experimental treatments groups

All goats were received one dose of 25 mg of progesterone (Progesvit®, Brovel, Mexico, i.m.) and then were randomly allocated to four experimental groups. Which received 24 h later contrasting i.m. doses of eCG: 1) eCG-200 (200 IU of eCG (Folligon®, Intervet, Mexico); n=10), 2) eCG-100 (100 IU of eCG; n=10), 3) eCG-50+50, (two 50 IU of eCG, 12 h apart, n=10), and 4) eCG-50 (50 IU of eCG; n=9); groups were balanced according to body weight and body condition score (Fig 1).

Evaluated response variables

During the 7 d subsequent to the eCG administration, estrus activity was evaluated during 15 min twice a day (0900 and 1700 h), through the use of 4 sexually active males, protected with an apron to prevent mating. The male goats were induced to sexual activity by a hormonal treatment based on testosterone using the method described by Luna-Orozco *et al*. (2012). Females were considered in estrus when they allowed to be mated and stayed motionless. To calculate the percentage of females in estrus, the total number of females in estrus divided into total of females treated x 100, was considered. The estrus latency was defined as the time elapsed between the eCG administration and the first mounting allowed by goats. The estrus duration was defined as the interval between the first and last mounting allowed by females. Natural mating was used only once to serve females during the first 12 h after the beginning of estrus.

The percentage of females that ovulated was calculated as the total number of females ovulating / total of females treated x 100. The ovulatory rate was defined as the total number of *corpus luteum* per experimental group, divided by the total number of goats that ovulated within the experimental group. These two variables which previously mentioned were measured 10 d after the eCG administration by means of a transrectal echography. The diagnosis of pregnancy was performed by a transrectal echography at 45 d after the administration of eCG (Fig 1).

Statistical analysis

The estrus latency, estrus duration and ovulatory rate were analyzed using an ANOVA and mean comparisons were performed by the Tukey Test, when required. The percentage of females in estrus, ovulation and pregnancy were analyzed using a contingency table and the chi-square test. All the statistical analyses were calculated using the statistical package SPSS 15.0. Significance level 0.05 was considered for all tests.

RESULTS AND DISCUSSION

On this study different doses of eCG were evaluated successfully induced estrus and ovulation in goats that were previously treated with intramuscular progesterone during seasonal transition reproductive period. The total percentage of females on estrus and ovulating in the experimental groups was 94.5% (37/39) without differences among experimental groups ($p>0.05$), (Table 1). The total percentage of time elapsed between the eCG administration and the estrus onset did not differ among the eCG-200, eCG-100 and eCG-50+50 groups (47.2 ± 10.86 h; $p>0.05$); yet, the largest value was observed in the eCG-50 group (94.86 ± 9.98 h; $p<0.05$). Besides, estrus induction occurred between 36 and 48 h after eCG administration in those experimental groups with doses equal or higher than 100 IU eCG (Fig 2). All the goats receiving doses equal or higher than 100 IU showed estrus and 70% of the females were synchronized from 36 to 48 h post-eCG administration 100% of ovulation was achieved in all the treated goats. Our results are consistent with those reported in other studies, similar doses of eCG and hCG were used in anestrous goats previously treated with intramuscular progesterone (Alvarado-Espino *et al.*, 2016; Carrillo *et al.,* 2019). The high

Table 1: Reproductive outcomes in Creole goats during the seasonal anestrus (transition period, during June) treated with either 200 (eCG-200), 100 (eCG-100), 50+50 (eCG-50+50) and 50 (eCG-50) IU of eCG. Doses were administered 24 h after the administration of 25 mg of intramuscular progesterone.

	Treatment group			
Variables	eCG-200	eCG-100	eCG-50+50	eCG-50
Goats in estrus (%)	10/10 (100)	10/10 (100)	10/10 (100)	7/9 (78)
Latency to estrus (h)	42 ± 3.69[b]	49.2 ± 2.8[b]	50.4 ± 3.49[b]	94.86 ± 9.98[a]
Duration of estrus (h)	32.4 ± 5.38	34.8 ± 4.18	39.6 ± 5.67	34.29 ± 9.23
Goats ovulating (%)	10/10 (100)	10/10 (100)	10/10 (100)	7/9 (78)
Ovulation rate (n, CL)	1.4 ± 0.22	1.5 ± 0.27	1.6 ± 0.22	1.43 ± 0.20
Goats pregnant (%)	7/10[a] (70)	7/10[a] (70)	8/10[a] (80)	2/9[b] (22)

[a,b,] Means with different superscripts within rows differ (P<0.05).

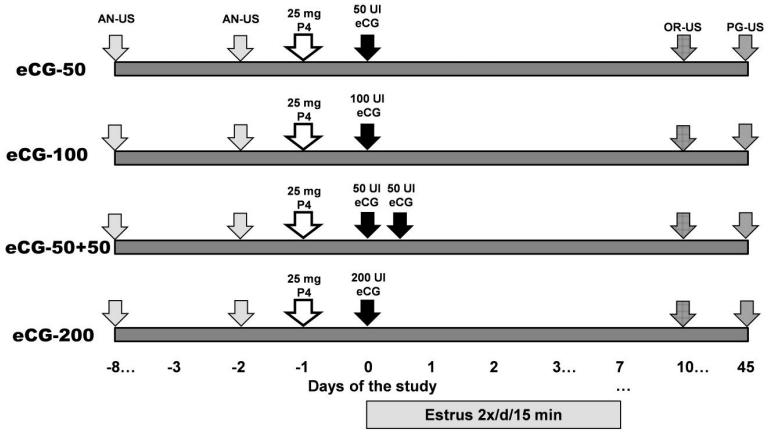

Fig 1: Schematic representation of the experimental protocols used to induce reproductive activity in Creole goats during the transition period (end of seasonal anoestrus, June). The goats were previously treated with intramuscular progesterone (P4), later, the experimental treatments, consisting in different doses (50, 100, 50+50 or 200 IU) of equine chorionic gonadotropin (eCG) were applied. The transrectal echography (US) was performed on days -8 and -2 in order to define the anovulatory (AN) state of the goats. At day 10 and 45, the ovulatory rate (OR) and pregnancy (PG) were confirmed, respectively.

number of estrous goats could have been due to the ability of eCG to activate the LH and FSH receptors in both the theca and the granulosa cells, respectively (Murphy, 2012). Such physio-endocrine scenario possibility induced follicular growth, increased in estradiol secretion and subsequently the presence of the estrus activity (De-Rensis and López-Gatius, 2014).

With respect to the high percentage of females ovulated it was observed that doses greater than 100 IU of eCG were enough to induce reproductive activity. Under such scenario, it is possible to suggest that eCG induced a positive feedback to estradiol, triggering the preovulatory LH suge, causing ovulation in most of the treated goats (Murphy, 2012; Carrillo *et al.,* 2019). It is worth mentioning that our result regarding goats ovulating are similar to those obtained with higher doses of eCG (300 IU; Vilariño *et al*., 2011). In fact, the high response to the low doses of eCG could have been promoted because this hormone has a relatively long-lasting bioactivity period, potentiating its effects on the ovarian activity, while inducing follicular growth and warranting ovulation (McIntosh *et al.*, 1975).

The total percentage of pregnant females for groups eCG-200, eCG-100 and eCG-50+50 was 73% (22/30; p>0.05). The lowest pregnancy rate (22%; 2/9; p<0.05) was recorded in the eCG-50 group (Table 1). In different studies, goats subjected to treatment protocols with progestogens + eCG, the pregnancy rate ranged from 61% (Martemucci and

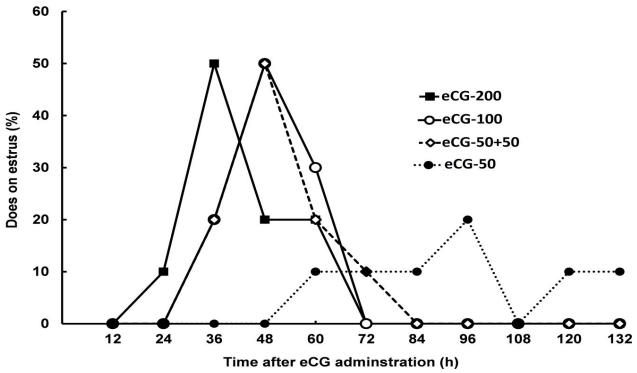

Fig 2. Time elapsed after the administration of the equine chorionic gonadotropin (eCG) until the appearance of estrus behavior in Creole goats managed under extensive conditions. All the females were treated at the end of the seasonal anestrus (June, 25ºN) with intramuscular progesterone and 24 h later, with different doses of eCG (200, 100, 50+50, 50 IU).

D'Alessandro, 2011) to 69% (Leboeuf *et al.,* 2003). In this study, no differences occurred among the experimental groups with doses equal or higher than 100 IU of eCG. Observing an important percentage of pregnant goats (73%), which confirms the interesting reproductive response obtained in goats treated with eCG. This could be explained because of the effect that eCG exerts upon both the theca and the granulosa cells, giving place to a bigger preovulatory follicle growth, as well as due to the eCG´s luteotrophic effect, which favors the *corpus luteum* growth and functionality (Thatcher *et al*., 2001). In fact, a *corpus luteum* with a bigger diameter increases its progesterone secretion (García-Pintos and Menchaca, 2016), favoring embryo implantation and the subsequent pregnancy.

The estrus and ovulatory responses of the goats treated with 50 IU of eCG was acceptable (78%) nonetheless, the dose of eCG and the asynchrony of the estrus activity could negatively influence the percentage of pregnant goats (22%). Possibly, this eCG dose was unable to reach the optimum threshold to correctly perform its different functions, such as a synchronized follicular growth, ovulation, and luteinization of the *corpus hemorrhagicum*. Indeed, a poor luteinization of the follicular cells could negatively affect the growth of the *corpus luteum* and, consequently, an abnormal progesterone synthesis.

Thus, dysfunctions of the *corpus luteum* promote embryonic losses and diminish pregnancy rate (Samir *et al.,* 2016). It would be convenient to perform more studies with these hormonal protocols and the intramuscular administration of progesterone plus eCG or hCG in different breeds and management systems as well as different periods of the seasonal anestrus, in order to be able to respond to these questions.

CONCLUSION

With the evidence provided by this study, we can conclude that the administration of intramuscular progesterone plus doses greater than 100 IU of eCG are effective hormonal strategies to inducing sexual activity of Creole goats, under subtropical extensive production systems during the transitional anestrous period (25ºN, June). The protocol described here makes the use of exogenous hormones more efficient, with reduce doses, decreasing expenses and is practical use by goat producers. This results can will apply to other ruminant industries.

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REFERENCES

Abecia, J.A., Forcada, F. and González-Bulnes, A. (2012). Hormonal control of reproduction in small ruminants. Animal Reproduction Science. 130: 173-179.

Alvarado-Espino, A.S., Meza-Herrera, C.A., Carrillo, E., González-Álvarez, V.H., Guillen-Muñoz, J.M., Ángel-García, O., Mellado, M. and Véliz-Deras, F.G. (2016). Reproductive outcomes of Alpine goats primed with progesterone and treated with human chorionic gonadotropin during the anestrus-to-estrus transition season. Animal of Reproduction of Science. 167: 133-138.

Carrillo, E., Meza-Herrera, C.A., Luna-Orozco, J.R., Delgado-Gonzales R.A., Gaytan-Aleman, L.R., Angel-Garcia, O., Veliz, F.G. and Contreras-Villarreal, V. (2019). Evaluation of out-of-season estrus induction protocols in progesterone-primed mix-breed dairy goats using eCG, GnRH and E2. Indian Journal of Animal Research. 53: 711-715.

Contreras-Villareal, V., Meza-Herrara, C.A., Rivas-Muños, R., Ángel-García, O., Luna-Orozco, J.R., Carrillo, E., Mellado, M. and Véliz-Deras, F.G. (2016). Reproductive perfor mance of seasonally anovular mixedbred dairy goats in duced to ovulate with a combination of progesterone and eCG or estradiol. Animal Science Journal. 87: 750-755.

Delgadillo, J.A. (2011). Environmental and social cues can be used in combination to develop sustainable breeding techniques for goat reproduction in the subtropics. Animal. 5: 74-81.

De-Rensis, F. and López-Gatius, F. (2014). Use of Equine Chori onic Gonadotropin to Control Reproduction of the Dairy Cow: A Review. Reproduction in Domestic Animals. 49: 177-182.

Duarte, G., Flores, J.A., Malpaux, B. and Delgadillo, J.A. (2008). Reproductive seasonality in female goats adapted to a subtropical environment persists independently of food availability. Domestical Animal Endocrinology. 35: 362-370.

FASS, (2010). Guide for the care and use of agricultural animals in agricultural research and teaching. (3rd Ed.), Federation Animal Science Society, Savoy, IL, USA.

García-Pintos, C. and Menchaca, A. (2016). Luteal response and follicular dynamics induced with equine chorionic gona dotropin (eCG) administration after insemination in sheep. Small Ruminant Research. 136: 202-207.

Leboeuf, B., Forgerit, Y., Bernelas, D., Pougnard, J.L., Senty, E. and Driancourt. M.A. (2003). Efficacy of two types of vaginal sponges to control onset of oestrus, time of preovulatory LH peak and kidding rate in goats inseminated with variable numbers of spermatozoa. Theriogenology. 60: 1371-1378.

Luna-Orozco, J.R., Guillen-Muñoz, J.M., De Santiago-Miramontes, M.d.l.A., García, J.E., Rodríguez-Martínez, R., Meza-Herrera, C.A., Mellado, M. and Véliz, F.G. (2012). Influence of sexually inactive bucks subjected to long photoperiod or testosterone on the induction of estrus in anovulatory goats. Tropical Animal Health and Production. 44: 71-75.

Manes, J., Campero, C., Hozbor, F., Alberio, R. and Ungerfeld, R. (2015). Vaginal histological changes after using intrav aginal sponges for oestrous synchronization in anoestrous ewes. Reproduction in Domestic Animals. 50: 270-274.

Martemucci, G. and D'Alessandro, A.G. (2011). Induction/synchronization of oestrus and ovulation in dairy goats with different short term treatments and fixed time intrauterine or exocervical insemination system. Animal Reproduction Science. 126: 187-194.

McIntosh, J.E.A., Moor, R.M. and Allen, W.R. (1975). Pregnant mare serum gonadotrophin: rate of clearance from the circulation of sheep. Journal of Reproduction and Fertility.

44: 95-100.

Murphy, B.D. (2012). Equine chorionic gonadotropin: an enigmatic but essential tool. Animal Reproduction. 9: 223-230.

Najmi, M.A., Aboulgasem, A.E., Jami, M.A. and Mokhtar, F.S. (2011). Indian Journal of Animal Research. 41: 63-64.

NAM, (2002). Guide for the care and use of laboratory animals. Co-produced by the National Academy of Medicine-Mexico and the Association for assessment and accrediation of laboratory animal care international. (1st. Ed.). Mexico, DF, Mexico.

Rahman, M.R., Rahman, M.M., Wan, K. and Abdullah, R.B. (2017). Effect of supplementation of hCG or GnRH on ovulation and subsequent embryo production of eCG superovulated goats. Indian Journal of Animal Re search. 51: 438-443.

Samir, H., Karen, A., Ashmawy, T., Abo-Ahmed, M., El-Sayed, M. and Watanabe, G. (2016). Monitoring of embryonic and fetal losses in different breeds of goats using real-time B-mode ultrasonography. Theriogenology. 85: 207-215.

Simões, J. (2015). Recent advances on synchronization of ovulation in goats, out of season, for a more sustainable production. Asian Pacific Journal of Reproduction. 4: 157-165.

Thatcher, W.W., Moreira, F., Santos, J.E.P., Mattos, R.C., Lopez, F.L., Pancarci, S.M. and Risco, C.A. (2001). Effects of hormonal treatments on reproductive performance and embryo production. Theriogenology. 55: 75-90.

Vilariño, M., Rubianes, E. and Menchaca, A. (2011). Re-use of intravaginal progesterone devices associated with the Short-term Protocol for timed artificial insemination in goats. Theriogenology. 75: 1195-1200.

Walkden-Brown, S.W., Restall, B.J., Scaramuzzy, R.J., Martin, G.B. and Blackberry, M.A. (1997). Seasonality in male Australian cashmere goats: Long term effects of castration and testosterone or oestradiol treatment on changes in LH, FSH and prolactin concentrations, and body growth. Small Ruminant Research. 26: 239-252.