**ABSTRACT**

Two separate studies were conducted aiming to determine reproductive performance (Study 1) and ovarian activity (Study 2) in goats treated with progestagen vaginal devices and equine chorionic gonadotropin (eCG). In study 1, estrus induction rate (EIR) and gestation percent (G%) were determined in multiparous (M) and primiparous (P) goats. In study 2, ovarian activity was determined in terms of follicle count (FC) and ovulation rate (OR) in puberal goats. In study 1, 28 P and 44 M goats were treated with progesterone vaginal devices (Conipred-CO) during 12 days and eCG doses of 0 (n = 11 P and 10 M), 200 (n = 11 P and 16 M) or 400 (n = 10 P and 14 M) IU, were administered upon device withdrawal. In study 1, EIR ranged from 45 to 90% and from 63 to 80% for the P and M goats, respectively, whereas G% ranged from 64 to 91% and from 78 to 80% for the P and M goats, respectively. The eCG dose increased significantly (P<0.05) EIR and G% in the P goats. In study 2, puberal goats were treated with Chronogest sponges during 12 days and doses of 0, 100, 200 or 400 IU of eCG (n = 5). The eCG dose did not affect FC, mean values found were 13, 12, 11 and 11 follicles, respectively, for each dose; whereas, OR was greater (P<0.05) in the goats treated with the 400 IU dose (2.87 ovulations), regarding the other doses (1.9 ovulations). In study 1, dose of eCG allowed improvements on EIR and G% in P goats, but not in the M goats; whereas, in study 2, eCG increased OR, in the highest dose group.

**Key words**: Synchronization of estrus, Gestation, Goats, Ovarian activity, Reproduction control.

**Short running title**: Synchronization of reproductive activity during the breeding season of goats.

**1. Introduction**

Animal food production for human consumption is a constant concern and a priority in several developing and underdeveloped countries (Delgado, 2003); likewise, human food consumption has been constantly increasing and thus protein needs (Delgado, 2003; Lima-Neto and Lima-Chalfun, 2018), and although, feeds of animal and plant origin differ in protein quality (Hoffman and Falvo, 2004; Pencharz et al., 2014), production of both kinds of proteins are required to meet the increasing demand imposed by the human population growth (Aiking, 2011; Lima-Neto and Lima-Chalfun, 2018). Thus, global animal food production requires to be increased in more efficient ways as well as the identification and implementation of reproductive technologies to maximize female reproductive efficiency and terminal productivity, need to be implemented.

Goats are seasonal poliestrous breeders and spontaneous ovulators, that follow a seasonal reproductive pattern, whose regulation depends on interactions of the central nervous sistem, the hypothalamus, the hypophysis with the genital tract (The hypothalamic-hypophyseal-gonadal [HHG] axis) and the environment (Goodman and Inskeep, 2006; Chemineau et al., 2010; Fatet et al.,2011; Luo et al., 2019); in addition, biological factors inherent to goats, such as breed, buck and goat effects and environmental factors, such as season and photoperiod (Chemineau et al. 2010; Fatet et al., 2011; Chemineau, 2015), are also involved. Such that, to induce year-around reproductive activity of the HHG axis and thus continuous reproduction, the design and implementation of reproductive management programs of males and females are required to increase overall reproductive efficiency and productivity (Delgadillo and Martin, 2015).

Implementation of male management strategies require exposing bucks to artificial photoperiod regimes to express continuous sexual behavior and semen production (Delgadillo et al., 2016b). Likewise, female management programs, require goats to be exposed to treatment protocols for synchronization and/or induction of estrus and ovulation (SI/EO), with (Abecia et al., 2012; Garza et al., 2015; Singh et al., 2019) or without (Delgadillo et al., 2012; Chemineau, 2015) exogenous hormones, to express reproductive behavior during the breeding or the anestrous season.

Furthermore, goat reproductive efficiency directly depends on the ovarian reserve, that is, the number of follicles that grow during each follicular wave of each estrous cycle, naturally ocurring or induced, in a such a way it is this follicle crop that determines the success of assisted reproductive technologies (Abecia et al., 2012; Luo et al., 2019; Singh et al., 2019); in adittion, direct relationship exists between the anti-mullerian hormone levels and the ovarian follicle reserve and embryo production (Monniaux et al., 2011).

In addition, reproduction is the most important component of animal production and the most important factor that determines productivity and development and despite remarkable advances in reproductive physiology (Hadgu and Fesseha, 2020), low conception rates and early embryo losses remain as major unsolved problems (Verma et al., 2012). To increase goat productivity, reproductive biotechnologies must be strategically used, such methodologies include SI/EO, artificial insemination (AI) and embryo transfer, among others (Hadgu and Fesseha, 2020; Holtz, 2005). Methodologies for goat SI/EO have been used for several years in different types of goats, using several treatment protocols, combined with AI (Agossou and Koluman, 2018; Atmoko et al., 2020; Fernández-Moro et al., 2008; Yotov et al., 2016); results for gestation percent have varied from 54 to 84% (Agossou and Koluman, 2018; Yotov et al., 2016).

The objectives of these studies were to determine reproductive performance (estrus induction rate, EIR and gestation percent, G%) and ovarian activity (follicle count, FC and ovulation rate, OR) in goats treated with progesterone or chronolone vaginal devices and equine chorionic gonadotropin (eCG).

**2. Materials and methods**

**2.1 Reproductive behavior in goats treated with progesterone vaginal devices and equine chorionic gonadotropin**

The first study was conducted at the Research Farm Ing. Herminio García González, Facultad de Ingeniería y Ciencias, Universidad Autónoma de Tamaulipas, Guémez, Tamps., México (23°56´26.5´´N, 99°05´59.9´´W and 193 m altitude; INEGI, 2017) from May through September, 2014). All procedures in this study were approved by the Animal Research and Experimental Ethics Committee of the Universidad Autónoma de Tamaulipas (Letter No. 2014-0016 of march 15, 2014).

**2.1.1 Animal management**

Goats were kept under feedlot conditions, in a shaded pen (20 m wide by 20 m long), and provided with clean and fresh water *ad libitum.* Goats were treated with vitamins A, D and E (Vigantol ADE Fuerte, Bayer, México), at the and dewormed with Ivermectina (Sanfer, México), with the recommended dosages from the laboratorios; goats were fed a maintenance ration (NRC, 2007), consisting of fresh citrus pulp (7-8% crude protein [CP], on a dry matter basis, González et al., 2014) *ad libitum*, plus one kg of a comercial concentrate (12% CP).

**2.1.2 Experimental protocol**

A total of 72 cycling goats were used (28 primiparous [P], 44 multíparous [M]) during the breeding season, cyclicity was assessed by monitoring overt estrus during 42 days prior to the start of the study, with apron-fitted intact bucks, goats were selected by having shown at least one overt estrus; goats in northern México regularly cycle from August through February (Delgadillo et al., 2016a). All goats selected were in a body condition score of 3 to 4 (range 1 thin to 5 obese, Burkholder, 2000; Gosh et al., 2019), with body weights of 23 to 40 kg. Reproduction in goats in northeast México is mostly regulated by season and photoperiod (Delgadillo et al., 2016a) and by nutritional status (Burkholder, 2000; Gosh et al., 2019).

Goats were initially primed with a double injection of prostaglandin F (PgF2α, 125 µg, Cloprostenol, Boviprost®, Lapisa®, México), administered intramuscularly (IM), 7 days apart, three days after the second PgF2α injection, goats were treated with vaginal devices (Cronipres CO®, 160 mg of progesterone, Biogénesis Bagó®, Argentina) for 12 days, upon device withdrawal, goats were randomly divided, considering type (P or M), as illustrated in Figure 1, into the eCG (Folligon®, Intervet®, México) treatments of 0 IU (n = 11 P goats and 10 M goats, 200 IU (n = 11 P goats and 16 M goats) and 400 IU (n = 10 P goats and 14 M goats); injections were administered IM in 2 ml of the diluent provided with Folligon. The methodology described herein and partial results weere presented in a mexican goat congress (Uvalle et al., 2013).

Estrus was detected during a 48 hour period, post-device withdrawal with apron-fitted bucks, aproximatelly 55 hours post-device withdrawal all goats were vaginally inseminated with a dose of fresh semen containing 200 million motile sperm cells; 8 days after AI, all goats were exposed to fertile bucks during 45 days, to breed the goats that did not become pregnant after the AI.

Pregnancy diagnosis was performed 60 days after vaginal device withdrawal using real time ultrasonography (Crilly et al., 2017). Data for EIR and G% were analyzed by means of Xi square tests, using SAS (Cody, 2015).

**2.2 Induction of ovarian activity in puberal does with Chronolone-impregnated vaginal sponges and equine chorionic gonadotropin**

The second study was conducted at the Area de Estudios Metabólicos of the Centro Universitario UAEM-Temascaltepec, Universidad Autónoma del Estado de México (CUT-UAEM), Temascaltepec, Edo. de México, México (19º2’42” N and 100º2’47” O, at 1,300 m of altitude, INEGI, 2015), from August through November, 2014. All procedures in this study were approved by the Animal Research and Experimental Ethics Committee of the Universidad Autónoma del Estado de México (Communication Letter No. 2014-03-120, of March 15, 2014).

**2.2.1 Animal management**

Twenty French Alpine puberal does of 6.5 months of age and 20.5 kg of body weight were allocated randomly and housed individually in 0.82 m by 1.24 m steel wire cages.

Does were fed a whole ration (12% CP), consisting of 40%:60% forage:grain mix, in the amount of 2.0 to 2.5 kg per head per day, to provide a maintenance ration (NRC, 2007). Fresh clean water was offered daily *ad libitum.*

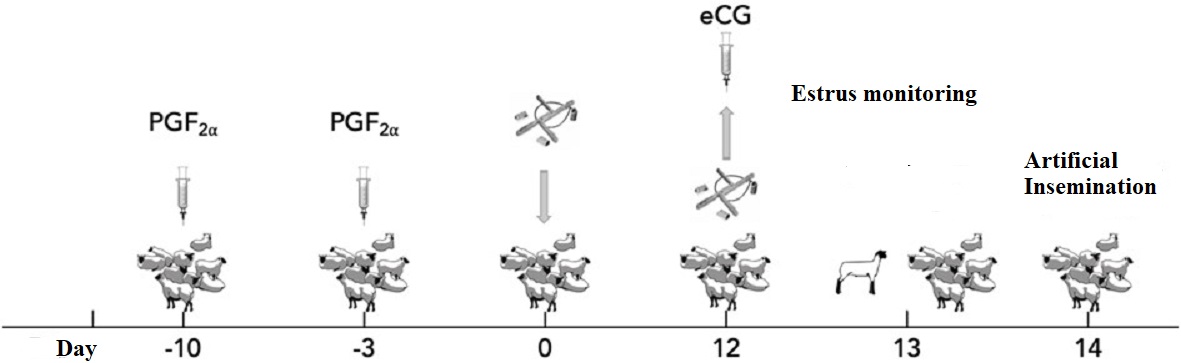


Figure 1. Diagram showing the time line used for the synchronization protocol for estrus in goats for study 1.

**2.2.1 Experimental protocol**

Does were treated for 12 days with vaginal sponges (20 mg chronolone, Chronogest® CR, Intervet®, México), at sponge withdrawal, does were randomly allotted to the following eCG treatments (n = 5), of 0, 100, 200 or 400 IU, administered IM, in 2.0 ml of the diluent provided with Folligon.

All does were non-cycling at the start of the study, as determined by the abscence of corpora lutea, assessed by real time ultrasound (Aloka 500, Tokyo, Japan).

Ovarian follicles were counted every 8 hours, during 96 hours, post-sponge withdrawal, by means of transrectal ultrasonography, using a real time ultrasound machine (Aloka 500, Tokyo, Japan), fitted with a 7.5 MHz lineal transducer, adapted to a rigid PVC handle, for proper ovarian exploration. The OR was assessed from 24 to 48 hours post-sponge withdrawal, based on dissappearance of the largest follicles.

Data analysis was performed using the SAS package (Cody, 2015). Follicular count data was evaluated using the repeated measures analysis, MIXED procedure (Cody, 2015), where each doe was considered as the random variable. The following equation was used:

*Yijk = μ + Ti + Goatj + Eijk*, where:

*Yij* represents the response of the *ijk*-th observation of size and follicular count,

*µ* represents the general mean,

*Ti* represents the effect of the *i*-th treatment of eCG dosage,

*Goatj* represents the *j*-th random effect of the doe, and

*Eijk* represents the random error.

Mean differences were assessed using the Tukeys test (α = 0.05, Steel and Torrie, 1989). The differences in percentage of goats ovulating by time period were detected by chi square analysis (α = 0.05, Steel and Torrie, 1989).

**3. Results**

**3.1 Reproductive behavior in goats treated with progesterone vaginal devices and equine chorionic gonadotropin**

Overall EIR was 70%, it was greater in the M goats (74%), than in the P goats (66%); the EIR increased significantly with eCG dose (P<0.05), in the P does, it increased from 45% (0 IU eCG) to 90% (400 IU eCG dose, Table 1). The EIR was greater (P<0.05) in the M goats treated with 0 (80%) or with with 400 IU (79%) of eCG, compared to M goats treated with 200 IU of eCG (62.5%).

Overall G% was 78.7%, the lowest and highest values were 63.6% (0 IU eCG dose group) and 90% (200 and 400 IU eCG dose groups), for the P goats. For the M goats, G% values were 80%, 79% and 79%, respectively, for the 0, 200 and 400 IU eCG dose groups (Table 1).

|  |  |  |  |  |
| --- | --- | --- | --- | --- |
| Table 1. Estrus induction rates (EIR) and gestation percentages (G%) in primiparous and multiparous goats treated with two doses of prostaglandin F2α, progesterone and equine chorionic gonadotropin (eCG), during the breeding season. | | | | |
| eCG (IU) | Primiparous goats | | Multiparous goats | |
| EIR (%) | Gestation (%) | EIR (%) | Gestation (%) |
| 0 | 45.5b | 63.6b | 80.0a | 80.0 |
| 200 | 63.6a | 90.9a | 62.5b | 78.8 |
| 400 | 90.0a | 90.0a | 78.6a | 78.6 |
| a,b Superscripts within columns denote significant differences (P< 0.05), IU: International units. | | | | |

**3.2 Induction of ovarian activity in puberal does with Chronolone-impregnated vaginal sponges and equine chorionic gonadotropin**

The ovarian response of the does treated with eCG doses is presented in Table 2. There was a significant effect (P< 0.05) of eCG dose on the numbers of small follicles (FC). The eCG dose did not affect the total FC, neither, the medium or the large follicle numbers.

The ovarian response expressed as the FC of the three classes of follicles is presented in Table 3, a significant effect (P0<0.5) was observed on the number of small follicles; in addition, an inverse relationship between the eCG dose and the number of small follicles was observed, the mean number of small size follicles decreased as the eCG dose increased (6.6 to 4.2, P<0.05).

|  |  |  |  |  |  |
| --- | --- | --- | --- | --- | --- |
| Table 2. Least squares means for the analysis of variance on the size of follicles in puberal does treated with differing doses of equine chorionic gonadotropin (eCG). | | | | | |
| Source of variation | DF | Follicular size (mm) | | | Total number of follicles |
| Small | Medium | Large |
| ≥ 1 a < 3 | ≥ 3 a ≤ 4 | > 4 |
| Dose of eCG | 3 | 48.85\* | 2.55ns | 0.49ns | 33.86ns |
| Time, hours | 6 | 475.19\* | 14.25\* | 2.16\* | 440.17\* |
| Interaction | 18 | 12.46 | 2.88 | 1.21 | 7.99 |
| Error | 17 | 15.82 | 4.42 | 1.01 | 13.11 |
| R square |  | 0.60 | 0.20 | 0.21 | 0.62 |
| Variation Coeff. |  | 72.53 | 40.96 | 93.75 | 30.98 |
| \* (P< 0.05), ns (P> 0.05); DF: Degrees of freedom. | | | | | |

No significant effects of eCG on the numbers of medium or large follicles were observed.

The size of the ovulatory follicle increased with eCG dose, however, the differences were not significant. The OR was significantly greater (P<0.05) for the does treated with the 400 IU eCG dose (2.9 ovulations), in relation to the three other eCG doses (1.9 ovulations).

|  |  |  |  |  |
| --- | --- | --- | --- | --- |
| Table 3. Effect of dose of equine chorionic gonadotropin (eCG) on the follicular size (Means ± SEM) in ovaries of puberal does treated with Chronogest vaginal sponges. | | | | |
| Dose of eCG | Follicular size (mm) | | | Total number of follicles per doe |
| Small | Medium | Large |
| ≥ 1 a < 3 | ≥ 3 a ≤ 4 | >4 |
| 0 IU | 6.6 ± 0.61a | 4.8 ± 0.32 | 1.0 ± 0.16 | 13.0 ± 0.56 |
| 100 IU | 6.1 ± 0.61ab | 5.1 ± 0.32 | 1.1 ± 0.16 | 12.3 ± 0.56 |
| 200 IU | 5.0 ± 0.61ab | 5.2 ± 0.32 | 1.0 ± 0.16 | 11.0 ± 0.56 |
| 400 IU | 4.2 ± 0.67b | 5.5 ± 0.36 | 1.2 ± 0.17 | 10.7 ± 0.61 |
| a,b Superscripts in a column denote significant differences (P< 0.05); IU: International units. | | | | |

|  |  |  |  |
| --- | --- | --- | --- |
| Table 4. Size of the ovulatory follicle, mean ovulation rate and mean time to ovulation (Means ± SEM) in puberal French Alpine does treated with equine chorionic gonadotropin (eCG) and Chronogest vaginal sponges. | | | |
| Dose of eCG | Preovulatory follicle diameter (mm) | Ovulation rate | Ovulation time from eCG treatment (hours) |
| 0 IU | 4.98 ± 0.20 | 1.89 ± 0.183b | 50.66 ± 2.17a |
| 100 IU | 5.18 ± 0.18 | 1.90 ± 0.166b | 46.90 ± 1.96ab |
| 200 IU | 5.11 ± 0.20 | 1.89 ± 0.183b | 41.33 ± 2.17bc |
| 400 IU | 5.44 ± 0.15 | 2.87 ± 0.137a | 39.70 ± 1.63c |
| a,b Superscripts within a column denote significant differences (P< 0.05); IU: International units. | | | |

The interval from eCG treatment to ovulation decreased with time (P<0.05), does treated with the 400 IU dose showed ovulation in less time (39.7 hours), whereas, the does of the three other dose groups required longer time (50.7 to 41.3 hours) to ovulation (Table 4).

Ovulation occurred faster in goats treated with higher eCG doses (P<0.05), 78, 82, 100 and 100% of does treated with 0, 100, 200 and 400 IU of eCG had ovulated by 36 hours after sponge withdrawal (Table 5).

|  |  |  |  |  |  |
| --- | --- | --- | --- | --- | --- |
| Table 5. Mean ovulation rate and percent of ovulation\* in puberal French Alpine does post-treatment with equine chorionic gonadotropin (eCG) and Chronogest vaginal sponges. | | | | | |
| Dose & time  after eCG | 0 IU | 100 IU | 200 IU | 400 IU | Ovulation rate |
| 24 hours | 0a | 27.3ª | 55.6b | 68.7b | 2.6±1.4ª |
| 36 hours | 77.8b | 54.6b | 44.4b | 31.3b | 2.0±1.6b |
| 48 hours | 22.2b | 18.2ª | 0a | 0a | 2.0±1.7b |
| a,b Superscripts within a column denote significant differences (P< 0.05); \* significant differences were detected by chi square analysis, IU: International units. | | | | | |

**4. Discussion**

Reproductive behavior in goats in response to protocols for SI/EO is expressed as estrus and reproductive performance and calculated as EIR, G% and prolificacy. The EIR is calculated as the proportion of goats showing estrus in relation to the goats treated and likewise, for the G% and kidding rate. Thus, productive and reproductive performance and productivity can be estimated for the goat herd.

Results found for EIR in the first experiment and with the SI/EO protocol used, indicated that goats during the breeding season can be synchronized for reproductive behavior, allowing goats to show estrus in acceptable rates (70% and higher), showing results similar to those found in other reports, also using similar protocols. Studies of Bukar et al. (2012), using a protocol based on PgF2α, progesterone and eCG, reported similar results; whereas other studies (Freitas et al., 1997) have reported higher values for EIR (93 to 95%); although, in these reports higher doses of eCG were used (500 IU), compared to the high dose used in these studies (400 IU), which resulted in the highest EIR (90%).

However, these results do not necessarily mean that a higher eCG dose will induce a higher EIR; in addition, Bukar et al. (2012) suggested that a dose of 300 IU is sufficient to induce a good estrus reponse, results that coincide with this report, since with a dose of 200 IU, a response of 63% was observed. Other results fall within the range of this report, which overall, vary from 65 to 94%; using doses of 400 IU of eCG; besides, goats treated with eCG and progesterone showed estrus in less time, regardless of eCG dose (Nava et al., 2010).

Several factors seem to affect the variation in EIR of goats in response to SI/EO protocols, one of such factors is the long half life of eCG in the circulation of the goat, it varies from 5 to 6 days (Cole et al., 1967) or from 40 to 52 hours (Martinuk et al., 1991), depending on the analytical method used. As a consecuence, this long half life would allow enough time for the eCG to act upon the ovaries and induce the follicles to secrete large quantities of estradiol; other advantage of estrus induction protocols using eCG, is that eCG is administered in a single injection. Furthermore, to fully assess the efficiency of SI/EO protocols, other influences must be considered, factors as nutrition, stress, socio-sexual, hormones and season (Goodman and Inskeep, 2006; Chemineau et al., 2010; Fatet et al., 2011; Delgadillo et al., 2012; Chemineau, 2015); besides, the type and quality of semen and the insemination method, are also important factors to consider (Faigl, et al., 2012; Luo et al., 2019); all these factors are responsible for modifying the reproductive performance of goats. In addition, goats that have been repeatedly treated with eCG may respond immunologically and produce antibodies against it and thus, become non responsive to hormonal protocols (Cordova et al., 2008).

Goats in medium to good body condition would respond well to SI/EO protocols and conversely, underfed goats that have lost weight, would show defficient gonadotropic and ovarian hormones secretion patterns and lower estrous behavior (Kandiel et al., 2010; Bukar et al., 2015).

In the study 1, overall G% ranged from 64 to 90%, studies of Kulaksiz and Daskin (2012) reported a G% of 67% and 53%, for P and M goats treated with sponges with fluorogestone acetate and eCG.

Other reports have concluded that G% varies depending on the SI/EO protocol and the AI method (Freitas et al., 1997; Cordova et al., 2008; Abecia et al., 2012; Faigl et al., 2012), with values for EIR and G% varying from 50% to 72%; whereas, others have reported values that varied from 59% to 88% (Freitas et al., 1997). Altogether, G% from this report and others reviewed here denote a wide range of variability, which may be due to environmental and biological factors inherent to the goat or to the biochemical and physiological properties of eCG and the nature of the SI/EO protocols.

In study 2, the number of small follicles decreased with eCG, the FC of medium size follicles increased as eCG increased, whereas, the number of large follicles did not change; in addition, the total FC decreased with eCG dose (13 to 11). Similar trends in FC have also been observed by others, Nogueira et al. (2015) reported FC values of 8 to 16; whereas, reduced numbers (4 to 6) were observed by Cruz et al. (2008), in Saanen and Anglo-Nubian goats and for other breeds of goats (Kandiel et al., 2010).

The variability in the follicular classes detected for the does in study 2, suggest that several components must be considered; initially, the numbers detected in each follicle class indicate the shifting in numbers and classes due to growth in response to eCG, secondly, these numbers also reflect the changes that occur during the follicular waves of a natural estrous cycle, and finally, these changes also reflect the growth rate of the follicles and at different stages, of the follicular phase of the induced cycle, in response to eCG. These results are also in agreement with some of the results previously published by others (Kandiel, et al., 2010; Bukar et al., 2015). Furthermore, the features of the follicular populations are somehow preserved in the goat during different physiological status, as reported by Muratza et al. (2019) and Nogueira et al. (2015), during the estrous cycle or during the early stage of gestation as reported by Kandiel et al. (2010).

In study 2, eCG affected follicular growth, as also observed by Uribe et al. (2010a), these researchers observed effects on the size of the dominant follicle, effect that persisted until the Graafian follicle stage, using similiar protocols. Murtaza et al. (2019) reported dominant follicle sizes varying from 6 to 8 mm in goats with 3 or 4 folllicular waves, during the estrous cycle; results that are similar (6.7 to 7.8 mm) to those reported by Nogueira et al. (2015); similar results also prevail by 35 days of pregnancy (Kandiel et al., 2012). The SI/EO protocols also influence follicle development and growth, a double PGF2α injection does not modify the features of the follicular waves (1, 2 or three waves), compared to the waves of the natural estrous cycle in ewes (Abecia et al., 2012; Uribe et al., 2010a).

An eCG threshold response exists for ovulation to increase, perhaps, beyond the natural OR, this threshold seems to be around the 200 IU dose, lower doses fail to increase OR, as suggested by these results; besides, there is also a wide range of variation in response to greater eCG doses; Freitas et al. (1997) and Mohammadi et al. (2010) reported values for OR of 1.8 and Murtaza et al. (2019) reported 1.6, compared to the one observed in this report (2.87 ovulations), using 400 IU (range from 1.89 to 2.87 ovulations for all doses).

The eCG therapy also affects follicle diameter and shortens the interval from withdrawal of progesterone treatment to ovulation, in a way that it indirectly reinforces the LH preovulatory peak, probably by means of an increase in estradiol synthesis and secretion by the growing follicle; in consecuence, estradiol concomitantly with endogenous GnRH cause a stronger positive feedback effect on the HHG axis to sooner induce the LH peak and thus shortening the interval to ovulation (Goodman and Inskeep, 2006; Uribe et al., 2010b); effects which are also suggested by these results. Furthermore, an eCG dose of 400 IU presumably induces a faster follicular growth, which is shown by the shorter mean time to ovulation and greater ovulation percentage of goats reached by 36 hours, as observed in the 200 and 400 IU doses group; these results also coincided with those of Uribe et al. (2010b).

In addition and as mentioned before, a greater eCG dose than the suggested threshold (200 IU) is required to obtain a greater OR, this, besides, to also obtain a greater conception and gestation rates, an OR and G% response relationship should be evaluated considering the eCG dosages, when using exogenous hormones or natural protocols and AI or when natural mounting programs are considered, after which, the kidding rate must also be taken into consideration.

The OR observed 24 hours (2.6 ovulations) post-eCG treatment was greater than the OR observed between 36 to 48 hours (2.0 ovulations), this effect is probably due to a faster follicle growth rate, higher estradiol secretion and higher LH peak induced by a higher eCG dose (eg: 400 IU). It is also possible, that a higher eCG dose would induce a greater FC to grow and hence a higher estradiol secretion rate and higher levels in the circulation.

In summarizing the endocrine events occurring during the follicular phase, which are similar to those of a synchronized estrus and should end in an ovulation, followed by a gestation and thus parturition; anatomical events that must be preceded by increase in PGF2α, followed by luteolysis and progesterone fall, estradiol rise, preovulatory gonadotropin rise and estrus (Goodman and Inskeep, 2006). However, evidence indicates that the follicular phase of the naturally occuring estrous cycles is longer (4 to 5 days, Nogueira et al., 2015; Murtaza et al., 2019), in relation to the duration of a follicular phase induced by a SI/EO protocol, plus eCG, and considering the time to ovulation determined here, which varied from 40 to 50 hours.

Another finding of study 2 was that goats with a single ovulation, presented smaller ovulating follicles, than goats having 2 to 4 ovulations, this effect is probably due to the eCG dose, since, an inverse relationship was observed between eCG dose and the mean time to ovulation, following eCG treatment; mean time to ovulation decreased as eCG dose increased from 0 to 400 IU; based on this evidence, it would seem feasible that a greater eCG dose would induce a faster growth rate and thus a larger size. In contrast, naturally cycling goats with OR of 1.6, show larger preovulatory follicles (7.2 mm, Murtaza et al., 2019), whereas Bukar et al. (2015) reported follicles varying from 7.0 mm to 9.6 mm in size; follicle sizes that are different to follicles detected here (4.9 mm to 5.8 mm); this difference may be due to the eCG dose, and further explained as perhaps also due to the number of follicles growing simultaneously on the wave initiated by eCG.

Furthermore, and as mentioned previously, preovulatory follicle diameter tended to increase with eCG dose; however, differences in OR were only significant in does treated with 400 IU, in which OR was highest (2.9 ovulations). The proportion of goats ovulating increased with eCG dose, furthermore, only 78% of the goats that did not receive eCG ovulated, whereas, 69% of the goats treated with 400 IU ovulated within 24 hours after eCG treatment.

**5. Conclusions and further comments**

Results found here for goats treated with progestagens and eCG, for SI/EO allowed to conclude that reproduction during the breeding season was synchronized and allowed goats to show reproductive behavior. However, to reach optimum reproductive efficiency, eCG needs to be adjusted, from the physiological and biological standpoints; which means, to successfully obtain synchrony of the endocrine and anatomical events during the follicular phase, that would result in a pregnancy and a subsequent parturition.

Thus, the SI/EO protocols employed here allowed to induce ovarian activity and reproductive behavior and performance in goats during the seasonal anestrus, which would allow in consecuence, the combined use of other technologies, as superovulation, embryo transfer and ovum pickup and *in vitro* embryo production (OPU-FIV).

In study 1, the use of a low to medium dose of eCG, as a dose of 200 IU, was sufficient to obtain reasonably a high G% values, in both cycling P and M goats; results that in consecuence would allow the use of technologies as AI and superovulation and embryo transfer. Results of study 2, indicated that the ovaries of goats can be estimulated with progestagens and eCG in puberal goats, to grow follicles at faster rates during an induced estrous cycle, results that can also be used for OPU-FIV.

However, information to fully make coincide the pharmacological effects of an eCG dose, with a biological, a physiological and a reproductive effect, is still awaiting to become a reality, which further, it should consistently yield sustainable and economically acceptable reproductive and productive parameters in goats.

**6. Acknowledgements**

The authors acknowledge the financial and infrastructure support provided by the central administration of both, FIC-UAT, in Cd. Victoria, Tamps., México, and the CU-UAEM Temascaltepec, in Temascaltepec, Edo. de México, México; and CONACYT México, for providing scholarships for graduate students.

**Disclosure statements**

The authors have no conflict of interest, intelectual or economic to declare.

**7. References**

Abecia, J. A., Forcada, F., González-Bulnes, A. 2012. Hormonal control of reproduction in small ruminants. Anim. Reprod. Sci. 130(3-4): 173-179.

Agossou, D. J., Koluman, N. 2018. The effects of natural mating and artificial insemination using cryopreserved buck semen on reproductive performance in Alpine goats. Arch. Anim. Breed. 61: 459-461.

Aiking, H. 2011. Future protein supply. Trends Food Sci. Technol. 22: 112-120.

Atmoko, B. A., Bintara, S., Maharani, D., Ibrahim, A., Budisatria, I. G. S. 2020. Estrous response of Etawah crossbred does on estrous synchronization using the prostaglandin f2α protocol. Earth Environ. Sc. 465:1-5, 012044.

Bukar, M. M., Malik, A., Hajarian, H., Kurnianto H. 2015. Selection for functional ovarian structures improves conception rates of oestrus synchronized crossbred Boer does. Alexandria J. Vet. Sci. 47(1): 32-37.

Bukar, M. M., Yusoff, R., Haron, A. W., Dhaliwal, D. K., Khan, M. A. G., Omar, M. A. 2012. Estrus response and follicular development in Boer does synchronized with fluorogestone acetate and PGF2α or their combination with eCG or FSH. Trop. Anim. Health Prod. 44(7): 1505-1511.

Burkholder, W. J. 2000. Use of body condition scores in clinical assessment of the provision of optimal nutrition. J. Am. Vet. Med. Assoc. 217: 650-654.

Chemineau, P. 2015. Una reflexión prospectiva sobre técnicas sostenibles para controlar la reproducción en mamíferos domésticos. Rev. Cient. Maskana 6(no. esp.): 31-48.

Chemineau, P., Bodin, L., Migaud, M., Thiery, J. C., Malpaux, B. 2010. Neuroendocrine and genetic control of seasonal reproduction in sheep and goats. Reprod. Dom. Anim. 45(s3): 42-49.

Cole, H. H., Bigelow, M., Finkel, J., Rupp, G. R. 1967. Biological half-life of endogenous PMS following hysterectomy and studies on losses in urine and milk. Endocrinology 81(4): 927-930.

Cody, R. P. An introduction to SAS® University edition. SAS Institute, Inc. Campus Drive, Cary, North Carolina, USA, SAS; 2015.

Córdova-Izquierdo, A., Córdova-Jiménez, M. S., Córdova-Jiménez, C. A., Guerra-Liera, J. E. 2008. Procedures to increase the reproductive potential in sheep and goat. Rev. Vet. (Arg.) 19(1): 67-79.

Crilly, J. P., Politis, A. P., Hamer, K. 2017. Use of ultrasonographic examination in sheep veterinary practice. Small Rum. Res. 152(july): 166-173.

Cruz, J. F., Teixeira, D. I. A., Rondina, D., Freitas, V. J. F. 2008. Dinâmica folicular ovariana em cabras em anestro após tratamento progestágeno. Rev. Bras. Saúde Prod. Anim. 9(4): 825-833.

Delgadillo, J. A., Duarte, G., Flores, J. A., Vielma, J., Hernández, H., Fitz-Rodríguez, G., Bedos, M., Fernández, I. G., Muñóz-Gutiérrez, M., Retama-Márquez, M. S., Keller, M. 2012. Control of the sexual activity of goats without exogenous hormones: Use of photoperiod, male effect and nutrition. Trop. Subtrop. Agroecosyst. 15(Supl. 1): S15-S27.

Delgadillo, J. A., Martin, G. B. 2015. Alternative methods for control of reproduction in small ruminants: A focus on the needs of grazing industries. Anim. Front. 5(1): 57-65.

Delgadillo S., J. A., Flores C., J. A., Véliz D., F. G., Duarte M., G., Vielma S., J., Poindron M., P., Malpaux, B. 2016a. Control de la reproducción de los caprinos del subtrópico mexicano utilizando tratamientos fotoperiódicos y efecto macho. Vet. Mex. 34(1):69-79.

Delgadillo, J. A., Vélez, L. I., Flores, J. A. 2016b. Continuous light after a long-day treatment is equivalent to melatonin implants to stimulate testosterone secretion in Alpine male goats. Animal 10(4): 649-654.

Delgado, C. L. 2003. Rising consumption of meat and milk in developing countries has created a new food revolution. J. Nutr. 133(Suppl.): 3907S-3910S.

Fatet, A., Pellicer-Rubio, M. T., Leboeuf, B. 2011. Reproductive cycle of goats. Anim. Reprod. Sci. 124(3-4): 211-219.

Faigl, V., Vass, N., Jávor, A., Kulcsar, M., Solti, L., Amiridis, G., Cseh, S. 2012. Artificial insemination of small ruminants - A review. Acta Vet. Hung. 60(1): 115-129.

Fernández-Moro, D., Veiga-Lopez, A., Ariznavarreta, A., Tresguerres, J. A. F., Encinas, T., Gonzalez-Bulnes, A. 2007. Preovulatory follicle development in goats following oestrous synchronization with progestogens or prostaglandins. Reprod. Dom. Anim. 43: 9-14.

Freitas, V. J. V., Baril, G., Saumande, J. 1997. Oestrous synchronization in goats: Use of fluorogestone acetate vaginal sponges or norgestomet ear implants. Anim. Reprod. Sci. 46(3-4): 237-244.

Garza A., A. J., González-Gómez, A., Vázquez-Armijo, J. F., Ledezma-Torres, R. A., Bernal-Barragán, H., Sánchez-Dávila, F. 2015. Status and implementation of reproductive technologies in emerging countries. Afr. J. Biotechnol. 14(9): 719-727.

González-Reyna., A., Lucero-Magaña, F. A., Zárate-Fortuna, P., Hernández-Meléndez, J., Ibarra-Hinojosa, M. A., Limas-Martínez, A. G., Martínez-González, J. C. 2014. Evolución del valor nutritivo de la pulpa de naranja fresca almacenada durante siete días. Zoot. Trop. 13(2): 159-164, Nota Técnica.

Goodman, R. L., Inskeep, K. E. 2006. Neuroendocrine control of the ovarian cycle of the sheep. In: Knobil, E., Neill, J. D., editors, Physiology of Reproduction, 3rd ed., Vol. 2, Baltimore: Elsevier Academic Press; 2006, pp. 2389-2446.

Hoffman, J. R., Falvo, M. J. 2004. Protein-Which is best. J. Sports Sci. Med. 3: 118-130.

Gosh, C. P., Datta, S., Mandal, D., Das, A. K., Roy, D. C., Roy, A., Tudu, N. K. 2019. Body condition scoring in goat: Impact and significance. J. Entomol. Zool. Studies 7(2): 554-560.

Hadgu, A., Fesseha, H. 2020. Reproductive biotechnology options for improving livestock production: A Review. Adv. Food Technol. Nutr. Sci. 8(1): 13-20.

Holtz, W. 2005. Recent developments in assisted reproduction in goats. Small Rumin. Res. 60(1-2): 95-110.

INEGI. 2016. Anuario Estadístico del Estado de Tamaulipas, México: Instituto Nacional de Estadística, Geografía e Informática, 365 p.

INEGI. 2015. Anuario Estadístico del Estado de México, México: Instituto Nacional de Estadística, Geografía e Informática, 380 p.

Kandiel, M. M. M., Watanabe, G., Abdel-Ghaffar, A. E., Sosa, G. A., Abou-El Roos, M. E. A., El-Azab, A. E. I., Li, J. Y., Manabe, N., Taya, K. 2010. Ovarian follicular dynamics and hormonal changes in goats during early pregnancy. J. Reprod. Develop. 56(5): 520-526.

Kulaksiz, R., Daşkin, A. 2012. Reproductive performance of primiparous and multiparous Saanen goats after laparoscopic intrauterine insemination: A field study. Turk. J. Vet. Anim. Sci. 36(2): 201-204.

Lima Neto, H. R. and Lima Chalfun, L. H. 2018. The increased consumption of animal products: Local aspirations-Global opportunities. Adv. Biotechnol. Microbiol. 9(3): 0065-0068.

Luo, J., Wang, W., Sun, S. 2019. Recent advances in reproduction for dairy goats. Asian-Australas. J. Anim. Sci. 32(8): 1284-1295.

Martinuk, S. D., Manning, A. W., Black, W. D., Murphy, B. D. 1991. Effects of carbohydrates on the pharmacokinetics and biological activity of equine chorionic gonadotropin in vivo. Biol. Reprod. 45(4): 598-604.

Mohammadi, A., Kohram, H., Gooraninejad, S., Yousefi, A., Motaghedi, A. 2010. Ovarian follicular dynamics during the interovulatory interval in Najdi goats. Afr. J. Biotechnol. 9(32): 5236-5239.

Monniaux, D., Baril, G., Laine, A. L., Jarrier, P., Poulin, N., Cognie, J., Fabre, S. 2011. Anti-müllerian hormone as a predictive endocrine marker for embryo production in the goat. Reproduction 142(6): 845-854.

Murtaza, A., Khan, M. I. R., Ahmad, W., Sohail, T., Mohsin, I., Shahzad, M., Tahir, M. Z., Ijaz, M. 2019. Follicular dynamics and changes in plasma estradiol-17β and progesterone concentrations during the estrous cycle in Beetal goats. Pak. Vet. J. 39(2): 193-198.

National Research Council (NRC). 2007. Nutrient requirements of small ruminants: Sheep, goats, cervids and new world camelids. Washington: National Academy Press, 365 p.

Nava-Trujillo, H., Chango-Villasmil, J., Finol-Parra, G., Torres-Rodríguez, P., Carrillo-Fernández, F., Maldonado-Suárez, J., Gil-Huerta, L., Akourki, A. 2010. Efecto de la dosis de eCG sobre la inducción del celo en cabras Mestizo luego de un tratamiento corto con Medroxiprogesterona. Rev. Cient., FCV, LUZ 20(2): 181-183.

Nogueira, D. M., Cavalieri, J., Gummow, B., Parker, A. J. 2015. Comparison of follicular dynamics and hormone profiles in Boer goats examined during the breeding and non-breeding seasons in the tropics of Queensland, Australia. Small Rumin. Res. 125(4): 93-100.

Pencharz, P., Jahoor, F., Kurpad, A., Michaelsen, K. F., Tomé, D., Weisell, R. 2014. Current issues in determining dietary protein and aminoacid requirements. Eur. J. Clin. Nutr. 68: 285-286.

Singh, B., Mal, G., Gautam, S. K., Mukesh, M. 2019. Reproduction biotechnology in goats. In: Singh, B., Mal, G., Gautam. S. K., Mukesh, M., editors. Advances in Animal Biotechnology. Switzerland: Springer Nature; pp. 301-308.

Steel, R. G. D., Torrie, J. H. 1989. Bioestadística: Principios y procedimientos. México: McGraw-Hill; 1989, pp.181-184.

Uribe V., L. F., Oba, E., Lenz S., M. I., Vélez M., M., Correa O., A. 2010a. Desarrollo folicular en ovejas durante el ciclo estral natural e inducido con prostaglandinas. Rev. Cient. FCV, LUZ 20(4): 417-421.

Uribe V., L. F., Lenz S., M. I., Osorio, J. H. 2010b. Resposta ovariana de cabras submetidas a implantes de progesterone seguidos de aplicaçôes de gonadotrofina coriõnica equina. Rev. Bras. Zootec. 39(6): 1214-1222.

Uvalle Martínez, N. B., González R., A., Hernández M., J., Zárate F., P., Briones E., Lucero M., F. A. 2013. Comportamiento reproductivo en cabras tratadas con prostaglandinas, progesterona y eCG. XXVII Reunión Nacional sobre Caprinocultura. Tlaxcala, Tlax., México, septiembre. 03-R, pp. 1-4.

Verma, O. P., Kumar, R., Kumar, A., Chand, S. 2012. Assisted reproductive techniques in farm animals: From artificial insemination to nanobiotechnology. Vet. World 5(5): 301-310.

Yotov, S., Atanasov, A., Karadaev, M., Dimova, L., Velislavova, D. 2016. Pregnancy rate in dry and lactating goats after estrus synchronization with artificial insemination and natural breeding (A field study). Bulg. J. Vet. Med. 19(3): 218-223.