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Ovarian response is not affected by the stage of seasonal anestrus or breed of goats when using a progesterone injection plus human chorionic gonadotropin-based protocol

Alan S. Alvarado-Espino[a], Alejo Menchaca[b], Cesar A. Meza-Herrera[c], Dalia I. Carrillo-Moreno[a], Santiago Zúñiga-García[a], Fernando Arellano-Rodríguez[a], Miguel Mellado[d], Francisco G. Véliz[a,*]

[a] *Universidad Autónoma Agraria Antonio Narro, Periférico Raúl López Sánchez y Carretera a Santa Fe, 27054, Torreón, Coahuila, Mexico*
[b] *Instituto de Reproducción Animal Uruguay, Fundación IRAUy, Cno. Cruz del Sur 2250, 12200, Montevideo, Uruguay*
[c] *Universidad Autónoma Chapingo, Unidad Regional Universitaria de Zonas Áridas, Bermejillo, Durango, 35230, Mexico*
[d] *Universidad Autónoma Agraria Antonio Narro, Departamento de Nutrición Animal, Calz. Antonio Narro 1923, 25084, Saltillo, Coahuila, Mexico*

A R T I C L E I N F O

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A B S T R A C T

The aim of this study was to evaluate the effect of the stage of seasonal anestrus and breed on ovarian response in non-estrous cycling goats using a progesterone (P4) injection plus human chorionic gonadotropin (hCG)-based protocol. In Experiment 1, non-estrous cycling local Mexican goats were treated with 20 mg of P4 plus 100 IU of hCG injections 24 h apart during April (early anestrus, $n = 13$) or June (late anestrus, $n = 12$). The estrous response, interval from hCG-to-estrus, and interval to ovulation were not affected by season ($P > 0.05$). In addition, the size of the follicle from which ovulation occurred and the size of the corpus luteum were not different between the two stages of seasonal anestrus ($P > 0.05$). In Experiment 2, the estrous response was compared between multiparous non-estrous cycling local Mexican ($n = 18$) and Alpine ($n = 19$) goats in which stage of the estrous cycle was synchronized using the same P4 + hCG protocol as in Experiment 1. Neither the onset of estrus nor the time of ovulation differed between breeds, and the estrus-to-ovulation interval also was similar for both breeds ($P > 0.05$). The diameter of the dominant follicle at the time of ovulation was similar between local and Alpine goats ($P > 0.05$). In addition, the pregnancy rate was not different for both local and Alpine goats ($P > 0.05$). In conclusion, results of this study indicate that the stage of seasonal anestrus or breed do not modify estrous and ovarian response in non-estrous cyclic goats synchronized with a P4 injection plus hCG-based protocol.

1. Introduction

Goats located in temperate and subtropical latitudes have a seasonal breeding pattern that results in a series of disadvantages for goat producers (Dardente et al., 2016). Currently, the hormonal protocols are perhaps the most common method used to synchronize time of estrus and ovulation for artificial insemination outside of breeding season (Menchaca and Rubianes, 2004). The conventional protocols consist of the insertion for 6–11 days of intravaginal sponges or controlled internal drug release (CIDR) devices containing

* Corresponding author.
E-mail address: velizderas@gmail.com (F.G. Véliz).

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progestogens or progesterone (P4), respectively. At the time of device removal, the females receive 200–500 IU of equine chorionic gonadotropin (eCG) intramuscularly (IM) (Arrébola et al., 2016; Vilariño et al., 2011). Alternatively, the use of a P4 injection plus hCG administration has been found to be suitable for controlling time of ovulation in non-estrous cycling goats during the anestrus-to-estrus transition period (Alvarado-Espino et al., 2016). This protocol consists of the IM injection of 20 mg of P4 followed by 100 IU of hCG administered 24 h later. With use of this simple protocol, an acceptable pregnancy rate is obtained when mating or artificial insemination occurs (Alvarado-Espino et al., 2016).

Even though these protocols can be used in controlling the estrous response and the time of ovulation, this response could be affected by factors such as season or breed (Corteel et al., 1988; Pierson et al., 2001; Ramukhithi et al., 2012). Pierson et al. (2001) reported that there was more variability in the interval from sponge withdrawal to the time of the luteinizing hormone (LH) pre-ovulatory surge release of LH during anestrus than during transition from the non-breeding to breeding season when Dwarf goats were treated with medroxyprogesterone (MAP) sponges and eCG. Baril and Vallet (1990) reported that super-stimulation of ovarian follicular development in Alpine goats resulted in greater variability in the onset of estrus after sponge withdrawal in females treated during the non-breeding season compared with females treated during the breeding season. In addition, regardless of the stage of the season, estrous response may be affected by breed and herd management (Lehloenya et al., 2005; Ramukhithi et al., 2012). In South African indigenous goats treated with the CIDR for 9 to 16 days plus 300 IU of eCG to synchronize time of estrus among animals, the proportion of goats in estrus was less and the length of induced estrus was shorter than in Boer goats (Ramukhithi et al., 2012). Because the efficacy of a fixed-time artificial insemination (FTAI) program depends on the efficacy of control of time of ovulation (Vilariño et al., 2011), these factors need to be further investigated.

Local Mexican goats have been extensively used for milk and kid production in rural marginal arid and semiarid areas in northern Mexico (Escareño et al., 2012; Wurzinger et al., 2013). As has occurred in other marginal conditions, reproductive programs are practically non-existent, and the use of a simple and low-cost protocol may improve reproductive efficiency especially when there is a desire to initiate breeding of females during the non-breeding season. An intensive milk production system is also used in this region where there is use of pure dairy goats for milk production that are managed in free-stall-intensive systems (Wurzinger et al., 2013). Although the hormonal protocols for estrous synchronization are usually utilized for estrous synchronization in these intensive systems, the development of easier-to-use and more affordable protocols that, when used, there is an acceptable fertility rate is also relevant to improve profitability. In addition, it is apparent that the responsiveness to non-photoperiodic modulatory effects or hormonal stimulus could have substantial effects that vary for the Alpine and local goats when there is use of a P4 plus hCG-based protocol for estrous synchronization. In addition, because there is no direct comparison between such breeds at the latitude where the present study was conducted, this study was undertaken to compare the response when there was use of the P4 plus hCG-based protocol in anestrous Alpine and local goats. The objectives of this study were to compare reproductive outcomes during both the early- and late-anestrous periods in local goats (Experiment 1) and to evaluate the response to the treatment in two different breeds (Experiment 2). In both experiments, the goats were submitted to a P4 injection plus an hCG-based protocol during the non-breeding season.

2. Materials and methods

All the methods and management of the experimental animals used in this study were in accordance with accepted guidelines for ethical use, care, and welfare of animals in research international (FASS, 2010) and national guidelines (NAM, 2002).

2.1. Experiment 1

Experiment 1 was performed during non-breeding season in the Comarca Lagunera, located in the northeast Mexico (26 °N). In this region the annual rainfall is 230 mm, and during this season the mean temperature is 23.5 °C (range −2 °C to 43 °C) and the day length is 13 h, 41 min at the summer solstice and 10 h, 9 min at the winter solstice.

The experiment was conducted using 27 multiparous, non-estrous cyclic local goats with 39.1 ± 4.4 kg of body weight (BW) and 2.3 ± 0.4 of body condition score (BCS; one emaciated and five obese (Atasever et al., 2015)). The goats were assigned to treatments using a randomized block design according BW and BCS when in early (April, $n = 13$) and late (June, $n = 14$) stages of the anestrous seasons. The months chosen for the study were based on the non-breeding season of local goats that were located at similar latitudes when assessments were made, which usually extends from late March to early August although, some females had spontaneous ovulations in May and June (Duarte et al., 2008). Anestrus was determined with two transrectal ultrasonic examinations (Aloka SSD 500; prostatic transducer 7.5 MHz) performed 8 days apart by an experienced operator, with the second assessment occurring immediately before the start of the experiments. The presence of corpus luteum (CL) was determined as evidence of estrous cyclicity in goats and any goats with a CL at either of these examinations was designated as estrous cycling and was not used for study. None of the goats examined in April were estrous cyclic (0/13, 0%), whereas two goats were estrous cyclic when examined in July (2/14, 14.2%, $P = 0.15$) and were removed from the study due to the presence of a CL. The female goats were housed in well-ventilated, open-lot, dirt floor pens with ample shade structures and 2.5 m of height in each pen. The goats were fed alfalfa hay and concentrates and had free access to water.

In both stages of anestrous, the goats were submitted to an estrous synchronization protocol that consisted of one IM injection of 20 mg of progesterone in oil adjuvant (0.4 mL; Progesterona, Zoetis, México) followed by 100 IU of hCG (0.1 mL; Chorulon, Intervet, México) 24 h later (day 0) (Alvarado-Espino et al., 2016). All injections were given in the morning. The estrous response and the interval from hCG to estrus was determined twice a day during a 15-minute period (performed from 0 to 5 days after hCG administration) by using two bucks with an apron to avoid complete mating. A female was considered to be in estrus when she stood to be mounted by the buck. The estrous response rate was calculated considering the number of goats in estrus/number of treated goats $\times 100$. The onset of estrus was defined as the time elapsed between hCG injection and the first mating. To determine follicular diameter, the goats were subjected to transrectal ovarian ultrasonography (7.5 MHz; Aloka SSD 500) at the time of the hCG administration, and then from the onset of estrus every 12 h until ovulation. Data for all follicles greater than 2 mm in diameter present in both ovaries were recorded and counted. Ovulation was defined as the collapse or disappearance of a large follicle, usually greater than 5 mm in diameter. On day 10 after hCG administration, all does were re-examined by transrectal ultrasonography to confirm the ovulation rate determined by counting the number of CL in both ovaries. The CL volume was calculated using the following formula: $V = 4/3 \times \pi \times r3$. Radius (r) was calculated using the formula, $r = (L/2 + W/2)/2$, where L = length and W = width.

2.2. Experiment 2

This experiment was conducted during non-breeding season in the Comarca Lagunera (May, 26 °N) on 17 local goats and 19 Alpine goats, all of which were multiparous non-estrous cycling females ranging between 2 and 5 years of age. Females of both breeds were submitted to the P4 injection plus hCG-based protocol described for Experiment 1. The local and Alpine goats weighed 37.0 ± 7.7 and 39.5 ± 6.3 kg ($P > 0.05$), and had a BCS of 2.3 ± 0.3 and 2.5 ± 0.4 ($P > 0.05$), respectively. Estrous detection, follicular diameter, ovulation, and number of CL were determined as described for Experiment 1. The females were mated by the males in the first 12 h after the onset of estrus. The diagnosis of pregnancy was made 45 days after mating by real-time, B-mode transrectal ultrasography.

2.3. Statistical analysis

The values for variables are expressed as the mean ± standard deviation (SD) or percentage. The interval from hCG to estrus and from hCG to ovulation, diameter of ovulatory follicle at ovulation, and ovulation rate were analyzed using the Student *t*-test for independent samples (TTEST of SAS, Institute Inc., Cary, NC, USA). The proportion of goats in estrus (over treated goats) and pregnant goats (over treated goats) were analyzed using the Genmod procedure of SAS. Each animal was considered as a single experimental unit.. The statistical level of significance was defined for a P value of 0.05.

3. Results

3.1. Experiment 1

The ovarian follicular population at the time of hCG administration was similar in April and June ($P > 0.05$; Table 1). The estrous response, interval to estrus, time of ovulation, and interval from estrus to ovulation were not affected by season ($P > 0.05$; Table 2). In addition, the size of the largest follicle at ovulation and the size of the CL did not differ among treatment groups ($P > 0.05$). The ovulatory rate was greater in goats treated in April (2.0 ± 0.0) than in those treated in June (1.6 ± 0.5; $P < 0.05$).

3.2. Experiment 2

The data for results of Experiment 2 are summarized in Table 3. The proportion of goats in estrus and those having ovulations tended to be reduced in the local (15/18, 83.3%) than in the Alpine goats (19/19, 100%; $P = 0.06$). Neither the onset of estrus nor the time of ovulation differed between breeds ($P > 0.05$). The interval from estrus to ovulation also was similar for both breeds ($P > 0.05$). In the local and the Alpine goats, 72% (13/18) and 100% (19/19) of females had ovulations between 72 and 96 h after hCG administration, respectively ($P > 0.05$). The diameter of the dominant follicle at ovulation tended to be smaller in the local (8.1 ± 0.7 mm) than the Alpine goats (8.7 ± 1.1 mm; $P > 0.05$). In addition, the ovulatory rate was greater for the local (1.6 ± 0.6) than the Alpine goats (1.3 ± 0.5; $P < 0.05$). The pregnancy rate in the local (10/18, 55.5%) and Alpine goats (15/19, 78.9%; $P > 0.05$) goats did not differ (Table 3).

Table 1
Number of small (2–3 mm), medium (4–5 mm), large (> 5 mm) and total (≥ 2 mm) follicles in non-estrous cyclic goats treated with a P4 injection plus human chorionic gonadotropin (hCG)-based protocol during April (early anestrus, $n = 13$) and June (late anestrus, $n=12$) at 26°N.

	Follicular diameter			Total follicles
	Small	Medium	Large	
April	1.9 ± 1.4	2.3 ± 1.2	2.2 ± 1.6	6.5 ± 1.7
June	2.4 ± 1.9	3.2 ± 1.8	1.4 ± 0.8	7.1 ± 1.3
P value	0.47	0.13	0.13	0.31

Data are presented as mean ± standard deviation (mean ± SD).

Table 2
Estrous response and time of ovulation in non-estrous cyclic goats treated using a P4 injection plus human chorionic gonadotropin (hCG)-based protocol during April (early anestrus) and June (late anestrus) at 26°N.

Variables	April	June	*P* value
Goats in estrus and ovulation (%)*	12/13 (92)	12/12 (100)	0.24
Interval from hCG to estrus (h)	58.0 ± 12.3	54.0 ± 6.2	0.33
Interval from hCG to ovulation (h)	91.0 ± 15.7	87.5 ± 9.7	0.51
Interval from estrus to ovulation (h)	33.0 ± 10.3	33.5 ± 12.9	0.91
Follicular diameter at ovulation (mm)	9.2 ± 0.7	9.3 ± 0.6	0.77
Ovulatory rate	2.0 ± 0.0	1.6 ± 0.5	< 0.05
Luteal tissue volume (mm^3)**	730.1 ± 233.4	740.0 ± 309.0	0.94

* All goats in estrus reach ovulation.
** 10 days after hCG.

Table 3
Ovarian response and fertility obtained in Alpine and local non-estrous cyclic goats using a P4 injection plus human chorionic gonadotropin (hCG)-based protocol during non-breeding season at 26°N.

Variables	Breed Local goats	Alpine goats	*P* value
Goats in estrus (%)	15/18 (83.3)	19/19 (100)	0.06
Interval from hCG to estrus (h)	58.2 ± 13.1	54.3 ± 7.3	0.32
Interval from hCG to ovulation (h)	96 ± 12.8	93.5 ± 7.6	0.50
Interval from estrus to ovulation	38.4 ± 11.2	39.1 ± 8.8	0.82
Diameter follicle at ovulation (mm)	8.1 ± 0.7	8.7 ± 1.1	0.17
Ovulatory rate	1.6 ± 0.6	1.3 ± 0.5	< 0.05
Pregnant goats (%)	10/18 (55.5)	15/19 (78.9)	0.12

4. Discussion

The results indicate that non-estrous cyclic local goats that were treated using a P4 injection plus hCG-based protocol during early and late anestrous season respond in a similar way in terms of estrous and ovulatory response because neither estrous response, interval to estrus, nor time of ovulation after hCG administration were affected by the stage of anestrous season. In addition, local and Alpine goats had a similar time of estrous onset and pregnancy rate when treated using this protocol during the non-breeding season.

In the present study, there was no difference in the estrous response between local goats treated during April and June. This result is consistent with those previously reported in dairy goat breeds in which there was imposing of estrous synchrony treatments using vaginal sponges and eCG or hCG during the non-breeding season or during the transition to estrous season. The estrous response in these previous studies ranged from 87% and 100% (Dogan et al., 2004; Fonseca et al., 2017; Souza et al., 2011). In the current study, there was no effect of the stage of anestrus on experimental outcomes because 92% of goats treated in April and 100% of those treated in June expressed estrus and had ovulations after treatment.

Results of some studies indicated that the interval to estrus and time of the preovulatory LH surge are more variable after estrous synchrony treatments have been imposed during the earlier part of anestrus than during the transition to estrus season in goats (Baril and Vallet, 1990; Pierson et al., 2001). The variability observed in the onset of estrus during the anestrous season has been attributed to differences in the rate of follicular growth during both the anestrous and breeding seasons (Pierson et al., 2001). In intact Saanen goats, it was reported that in the anestrous season, the rate of LH pulse release was less, and as the natural breeding season approached, frequency of LH pulse release increased, resulting in a greater rate of follicular growth at the end of anestrus (Chemineau et al., 1988). In the present study, the lack of an effect of stage of the anestrous period at time of imposing estrous synchrony treatments on ovarian response may be because one injection of P4 and hCG was adequate to synchronize the stage of follicular wave development and induce ovulation when treatments occurred in both phases of anestrus. Furthermore, the local goats at this latitude are considered to be a breed that is moderately to minimally affected by stage of the anestrous seasonal, thus these goats are considered to have a "shallow" anestrus (Mellado et al., 2014). There, however, may be other cues that affect the length and "depth" of anestrus (Urrutia Morales et al., 2016). Indeed, at the onset of treatment, number of follicles larger than 5 mm in diameter was similar in April compared with June, and there was no difference in the proportion of estrous cyclic goats (0/13 and 2/14, respectively; $P > 0.05$), which could indicate that goats with a similar ovarian status will respond to a P4 injection plus a hCG-based treatment protocol in early and late anestrus in a similar manner.

In previous studies, breed of goats appeared to have an effect on the estrous response, length of estrus, or ovulatory rate (Lehloenya et al., 2005; Nuti et al., 1987). Results of the present study, however, indicate that at the latitude the present experiment was conducted, both local and Alpine goats had a similar estrous and ovarian response when there were treatments using a P4 injection plus hCG-based protocol. This lack of breed effect is considered to be the case because in Experiment 1 ovulations occurred during a short period of time of 24 h in both breeds (i.e., 72–96 h after hCG administration). In addition, the mean diameter of follicles at ovulation and the ovulatory rate were similar to those reported in dairy goats where there was induction of estrus during the anestrous season (Fonseca et al., 2017; Souza et al., 2011). Evidence exists that the efficacy with use of different methods for the control of reproduction in small ruminants depends on the “depth” of anestrus that occurs with the breed (Corteel et al., 1988). Breeds originating from temperate regions had a greater sensitivity to photoperiodic changes during the year (Amoah et al., 1996). Nonetheless, in Alpine goats raised in tropical and subtropical regions, where changes in photoperiod are not significant, there was very little ovarian function throughout the year (Silva et al., 1998). Because there was no difference in the ovarian response after hormonal stimulation in the present study, it might be that in goats at subtropical latitudes, photoperiodic inhibition of reproduction in Alpine goats is less pronounced (Delgadillo and Vélez, 2010; Silva et al., 1998). Thus, Alpine females should respond in a similar way as the local goats, and seasonal period appears to not be a limiting factor in goats subjected to estrous synchronization protocol used in the present study.

5. Conclusion

In conclusion, in the conditions where the present study was conducted, the results indicate that the stage of the anestrous period does not affect the estrous and ovarian responses in non-estrous cyclic goats where there was estrous synchrony induced using a P4 injection plus hCG-based protocol. Furthermore, local goats and Alpine goats respond in a similar way to hormonal treatments in terms of estrous response, time of ovulation, and pregnancy rate.

Conflict of interest

The authors declare that there is no conflict of interest.

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