Effect of two routes of administration of human chorionic gonadotropin upon oestrus induction and reproductive outcomes in adult acyclic mix-breed goats

R. Rodríguez-Martínez, C. A. Meza-Herrera, K. I. Tapia-Robles, A. S. Alvarado-Espino, J. R. Luna-Orozco, C. Leyva, M. Mellado and F. G. Véliz-Deras

1. Introduction

While reproductive technologies represent a unique opportunity for goat producers to improve the breeding value of their flocks, several efforts have been made to develop protocols to induce and synchronize oestrus and ovulation during both the breeding and non-breeding seasons (Simoes 2015). Improvements in the design of oestrus induction protocols have allowed to expand the out-of-season use of artificial insemination, inseminating goats at specific times, while achieving pregnancy rates comparable to those obtained with the use of visual detection of oestrus (Menchaca & Rubianes 2004; Simoes 2015). Common protocols to induce oestrus in acyclic goats include the combined or single use of gonadotropins, gonadotropin-releasing hormone (GnRH), progestins and prostaglandins (Esteves et al. 2013; Contreras-Villarreal et al. 2016; Sen & Onder 2016).

Nonetheless, in marginal-grazing production systems, the use of expansive reproductive technologies is not attractive to most producers because of the high costs of different drugs required to control the oestrus cycle (Gonzalez-Bulnes et al. 2011). Hence, efforts to simplify oestrus induction protocols with the concomitant cost reduction are essential in order for goat-keepers to use cheap, simple and effective protocols for out-of-season oestrus induction. One of the strategies to accomplish that is the elimination of progestins and prostaglandins from such protocols (Menchaca & Rubianes 2004). In acyclic goats, the use of human chorionic gonadotropin (hCG) alone could be an effective strategy to induce reproductive activity. Besides, although follicular development can be observed during the anoestrus period, such growth is quite limited, hampering the ovulatory stage (Sarath et al. 2012). Recently, Alvarado-Espino et al. (2016) reported that a single injection of 100 IU of hCG was able to induce oestrus response and ovulation in Alpine goats during seasonal anoestrus. Besides, despite the specific anastomoses involving the vulva, vagina, uterus and ovaries, it is still elusive if such angioarchitectural scenario may allow a more efficient action of drugs injected throughout the intravulvosubmucosal (IVSM) than the intramuscular (IM) route (Meira et al. 2006). This trial aimed to evaluate the use of a single shot of hCG administered by two different routes, either IM or IVSM, upon oestrus induction and some other reproductive outcomes in acyclic mix-breed goats.

2. Material and methods

2.1. General

All the methods and management of the experimental units used in this study were in strict accordance with accepted guidelines for ethical use, care and welfare of animals in research at the international (FASS 2010), national (NAM 2002) and institutional levels, with approval reference number UAAAN/UL/1330-8241-2868.
2.2. Location and animal management

The present study was conducted in a commercial herd managed under extensive conditions during April–May, the natural non-breeding season for goats in Northern Mexico (25° ||N, 1,140 m altitude) (Mellado et al. 2014). While the annual average for temperature is 23.5°C (range –2 to 43°C), the annual relative humidity ranges between 28% and 81%. The annual rainfall in this area is 230 mm, and the day length is 13 h, 41 min at the summer solstice and 10 h, 9 min at the winter solstice. Goats grazed on open highly degraded rangeland typical of the Chihuahuan desert year round, driven by a herdsman (8 h per day; 1000–1800 h). Animals were penned near the household at night without access to feed and water. Goats kidded between September and December. Under the traditional management of these marginal production systems, goats receive neither food supplements nor minerals throughout the year, and were not treated against internal and external parasites, neither received any preventive vaccine management. To have a better control of the experimental units during the trial, goats were housed in three 4 × 4 m roofed pens and were offered alfalfa hay ad libitum plus 200 g concentrate with 14% crude protein content.

2.3. Confirmation of anoestrus status and experimental procedures

Pluriparous non-cycling mix-breed goats (Native × Alpine-Saanen dairy breeds) were used in the study. Averages for live weight (LW) and body condition score (BCS) were 39.6 ± 2.18 and 1.71 ± 0.2 kg. BCS was determined by tactile appraisal of fat in the sternum and lumbar vertebrae; scale 1–5 (Russell et al. 1969). In order to confirm anovulatory status, prior to the onset of the experimental treatments, 30 goats underwent transrectal ultrasonographic scanning conducted by a single experimenter, on days −15, −10 and −5 regarding the onset of the experimental treatments, using an Aloka 500 with a 7.5-MHz human prostate transducer (linear array; Corometrics Medical Systems, Inc., Wallingford, CT). During the ultrasonographic scanning, does were placed in the standing position and faeces were removed at the moment of the scanning. A coating of carboxymethylcellulose was applied to the vulva by theaprioned operator, on days 15, 10 and 5 to reduce error, only diameter and position in both ovaries of those follicles >3 mm in diameter were recorded. Follicles of both the ovaries were counted, measured and classified as small (3–4 mm), medium (4.5–5.4 mm) and large (>5.5 mm). Diameter and position of corpora lutea were also recorded. Pregnancy was detected at 45 days post-breeding throughout transrectal ultrasound (Aloka SSD 500, 7.5 MHz linear transducer).

Response variables consider oestrus (%), ovulation (%), ovulation rate, dominant follicle diameter (>5.5 mm), interval of onset of oestrus-to-ovulation (h), oestrus length (h), pregnancy (%) and litter size. The does were considered in oestrus when they were mounted by the teaser bucks. Oestrus duration was defined as the time between the first and the last accepted mount, within the same oestrus period. Ovulation rate was determined by the appearance of the dominant follicle on the image in the prior ultrasound examination and confirmed by the presence of corpora lutea on the surface of both ovaries of all goats. Additional variables recorded were pregnancy rate at day 45 post-oestrus, the interval from the start of treatment to oestrus and prolificacy, which consider the number of kids born per kidding doe.

2.4. Ultrasonographic measurements of ovarian structures

Upon oestrus onset and once bred by the males, the goat’s ovarian follicular activity was monitored up to day + 5 throughout daily transrectal ultrasonographic examinations. Ovarian images were obtained with a B-mode scanner (Aloka SSD 500, Overseas Monitor Corp. Ltd, Richmond, BC) equipped with a 7.5-MHz transducer (1.6 cm, diameter, 35 cm, length). The transducer was manipulated externally, with the doe in the standing position on a raised wooden platform and placed in a narrow chute. The image on the scanner was frozen at 1.5×; at this magnification, ovarian follicles around 1 mm were detected; but in order to reduce error, only diameter and position in both ovaries of those follicles >3 mm in diameter were recorded. Follicles of both the ovaries were counted, measured and classified as small (3–4 mm), medium (4.5–5.4 mm) and large (>5.5 mm). Diameter and position of corpora lutea were also recorded. Pregnancy was detected at 45 days post-breeding throughout transrectal ultrasound (Aloka SSD 500, 7.5 MHz linear transducer).

FM (n = 9; 39.8 ± 2.05 kg LW, 1.72 ± 0.2 BCS); (2). 100 IU hCG (0.1 mL) IVSM (n = 9; 39.0 ± 2.29 kg LW, 1.72 ± 0.2 BCS) and (3). Saline (0.5 mL) IM injection (CONT; n = 9; 39.9 ± 2.3 kg LW, 1.71 ± 0.2 BCS). The dose of 100 IU of hCG was based on previous results generated by our group (Alvarado-Espino et al. 2016). Ovarian structures, either follicular or luteal, were monitored by transrectal ultrasonographic scanning during 5 days post-hormonal treatments.

Twelve hours after hCG administration, entire aproned bucks of proven libido were introduced to each experimental group in a proportion of 1:9. The does from all groups were exposed to the bucks at the same time, two times per day (0600 and 1800 h × 15 min) (Angel-Garcia et al. 2015). Does were considered in oestrus when they were mounted by the aproned bucks (Chemineau et al. 1992). Then, 12 h after, the confirmed-oestrus goats were exposed to one sexually active buck within treatment, with previous sexual experience and proven libido and fertility, treated with testosterone (testosterone cypionate IM, 25 mg, every 3 days × 3 weeks prior to the goat’s hormonal treatments; Luna-Orozco et al. 2012). Both oestrus detection and the experimental breeding were performed during a 15-day post-treatment period.
2.5. Statistical analyses

The continuous variables time interval from treatment to oestrus onset, and follicle diameter, considered a general linear model with treatment as the main effect (PROC GLM, SAS Inst. Inc., Cary, NC). Mean values were compared according to a cut-off point of 0.05. No interactions were tested. Data on oestrus response, ovulation rate, pregnancy rate and kidding rate were analysed by categorical procedures using the PROC frequency procedure and general linear model procedures of SAS with the logit link function. The Wilcoxon Rank Sum test (the non parametric on way Wilcoxon Rank Sum test of SAS) was used to analyse prolificacy. Statistical differences among treatments were considered to be significant at $P < .05$.

3. Results and discussion

None of the goats in the control group presented oestrus, ovulation or growth of ovarian structures (Table 1), so reproductive variables were only compared between the hCG-treated groups. Certainly, while the CONT-goats never depicted neither oestrus nor ovulation (0%) ($P < .05$), the oestrus and ovarian response from the IM and IVSM groups was similar (89% vs 78% and 89% vs 68%, respectively ($P > .05$)). Results of our study confirm such hypothesis that the combination of P4-primed plus hCG in the present study was successful in inducing oestrus, regardless of the administration route, with quite notable reproductive outcomes, suggesting that this simple protocol, regardless of the administration route, not only activated the ovarian steroidogenesis pathway by the dominant follicles to induce oestrus behaviour, but also induced a per-ovulatory gonadotropin surge, which, at the end, lead to ovulation and luteinization.

The oestrus behaviour percentage observed in the present study is in agreement with that reported in other trials using a combination of fluorogestone acetate, prostaglandin F2α (PGF2α), equine chorionic gonadotropin (eCG) or follicle stimulating hormone (FSH) (Greiling & Van der Nest 2000; Fonseca et al. 2005a; Bukar et al. 2012), or more simple protocols (López-Sebastían et al. 2007). The proportions of does showing oestrus, during a 108-h period after the application of hCG, are shown in Figure 1. The interval from P4-primming to the beginning of oestrus was similar between groups of goats receiving the hCG treatment (IM, 63 ± 5.3 and IVSM, 54.9 ± 4.3 h; $P > .05$). This interval, ~60 h, is close to other reports using medroxyprogesterone acetate for 6 and 9 days, eCG and PGF2α (Greiling & Van Niekerk 1990; Fonseca & Torres 2005; Fonseca et al. 2005a). It has been hypothesized that hCG injection in the vulvar submucosa would enhance the action of this hormone by acting more rapidly on the ovaries, delaying the absorption or slowing the metabolism of this hormone and extending its action, while allowing a reduction in the minimal effective dose (Meira et al. 2006). Since the application of hCG via IVSM is much more difficult to apply than the IM injection, our results point out to the IM route as a better option from a practical standpoint to widespread its use under field conditions.

The overall number of follicles of different sizes prior to experimental treatments (~15, ~10 and ~5) were pooled and are presented in Table 2 as ‘~5 days’; the observed small, medium and large follicles across treatments are presented for days 1 and 5, also in Table 2. The number of small, medium and large ovarian follicles was not different ($P > .05$) among goats at ~15, ~10 and ~5 days (5.8 ± 1.0, 1.4 ± 0.2 and 2.0 ± 0.5 mm, respectively) and was indicative of a reduced ovarian activity without follicles capable of achieving ovulatory status. These follicles have been described by Ginther and Kot (1994) as the dynamic pool of antral follicles that develop and regress rather than be part of a cohort of gonadotrophin-dependent follicles, one or more of which might grow to the pre-ovulatory size and ovulate. Then, the remaining follicles undergo structural and functional atresia at various stages of development, under the influence of gonadotrophins (Gonzalez-Bulnes et al. 2005; Simoes et al. 2006). Nonetheless, after hCG administration (days 1–5), the number of small follicles was decreased (IM: 2.9 ± 0.7 and IVSM: 3.3 ± 0.6 for day 1 and IM: 2.8 ± 0.4 and IVSM: 2.3 ± 0.4 for day 5), while no differences occurred with respect to the number of medium (IM: 2.4 ± 0.7 and IVSM: 1.5 ± 0.3 day 1) and large-sized follicles (IM: 2.3 ± 0.3 and IVSM: 2.6 ± 0.7 for day 5) between the hCG groups. Interestingly, however, by day 5, while the control group depicted an

### Table 1.

Reproductive outcomes in multiparous non-cycling mix-breed goats (Native × Alpine-Saanen) during the anoestrus season in response to a single administration of 100 IU hCG through the intramuscular (IM) or intravulvar submucosal (IVSM) administration route, Northern Mexico (25°N).

| Variable                   | IM (89% / 9/9)        | IVSM (78% / 7/9)       | CONT (0% / 0/9)  |
|----------------------------|-----------------------|------------------------|------------------|
| Does in oestrus (n)        | 63 ± 5.3*             | 54.9 ± 4.3*            | –                |
| Interval from hCG to oestrus (h) | 30.7 ± 4.1*          | 36 ± 3.9*              | –                |
| Duration of oestrus (h)    | 33.6 ± 5.6*           | 34 ± 4.0*              | –                |
| Does ovulating (n)         | 89% (8/9)             | 68% (6/9)              | 0% (0/9)         |
| Interval from hCG to ovulation (h) | 100.8 ± 2.6*        | 90 ± 2.8*              | –                |
| Interval from oestrus onset to ovulation (h) | 7.6 ± 0.2*         | 7.8 ± 0.2*             | –                |
| Diameter of dominant follicle (mm) | 1.8 ± 0.14*         | 1.7 ± 0.23*            | –                |
| Numbers of ovulation       | 66% (6/9)             | 55% (5/9)              | –                |
| Conception rate (n)        | 1.5 ± 0.2*            | 1.6 ± 0.2*             | –                |

Note: Values are means ± s.e.m.

*Mean values with different superscripts among columns, within variable, differ ($P < .05$).

Figure 1. Percentage of oestrus behaviour across time (h) after hCG treatment (time 0) in multiparous non-cycling mix-breed goats (Native × Alpine-Saanen) during the anoestrus season. Goats received a single administration of 100 IU hCG through the intramuscular (IM), intravulvar submucosal (IVSM) or Control (CONT) administration route, Northern Mexico (25°N).
increased number of small follicles, those goats treated with hCG depicted a reduction in the number of small follicles as well as a concomitant increase in the number of large follicles, irrespective of the hCG administration route (Table 1).

Therefore, goats receiving the hCG treatment depicted the greater population of larger follicles, suggesting an increased follicular steroidogenesis which apparently augmented the plasma oestriadiol concentration, and promoted an increased oestrous behaviour in the hCG-treated goats, similar to that reported by Fonseca et al. (2005b). Apparently, the rise in gonadotropin induced the gonadotrophin-dependent follicles to emerge from a pool of gonadotrophin-responsive follicles (Viñoles et al. 1999). After emergence, one or several of these follicles are able to achieve an ovulatory status by expressing luteinizing hormone receptors on their granulosa cells and become independent of FSH influence. The fact that exogenous hCG increased the number of large follicles indicates that hCG is a feasible mechanism to stimulate folliculogenesis in anoestruis goats, and that these potentially ovulatory follicle(s) continued to develop and ovulate. Ovulation occurred when follicles reached nearly 8 mm in diameter (Table 1). No differences (P > 0.05) in follicle diameter were observed between groups treated with hCG (IM: 7.6 ± 0.2 and IVSM: 7.8 ± 0.2). The mean diameter of the dominant follicle was similar to that reported in previous studies carried out under similar conditions (Lehloenieya et al. 2008; Vazquez et al. 2010), and was indicative of fertility as confirmed by the observed pregnancy rate (IM: 66% and IVSM: 55%) and litter size for both IM and IVSM groups (1.5 ± 0.2 and 1.6 ± 0.2, respectively) (Table 1).

To conclude, our results indicate that a single injection of 100 IU hCG may be a simple and effective way of inducing fertile oestrus and suitable reproductive outcomes, the last irrespective of the administration route, either IM or IVSM. These simple methods generated important results regarding not only oestrus induction and ovulation, but also interesting out-of-season values for pregnancy rate, kidding rate and litter size, particularly since the control group was unable to depict oestrus and become pregnant. It is important to highlight that such reproductive outcomes were obtained in a genotype mainly based in quite seasonal mix-breed dairy breeds (Native × Alpine-Saanen dairy) under marginal conditions.

**Disclosure statement**

No potential conflict of interest was reported by the authors.

**References**

Alvarado-Espino AS, Meza-Herrera CA, Carrillo E, González-Álvarez VH, Guillen-Muñoz JM, Ángel-Garcia O, Mellado M, Véliz-Deras FG. 2016. Reproductive outcomes of Alpine goats primed with progesterone and treated with human chorionic gonadotropin during the anestrous-to-estrous transition season. Anim Reprod Sci. 167:133–138. doi:10.1016/j.anireprosci.2016.02.019

Ángel-Garcia O, Meza-Herrera CA, Guillen-Muñoz JM, Carrillo-Castellanos E, Luna-Orozco JR, Mellado M, Véliz-Deras FG. 2015. Seminal characteristics, libido and serum testosterone concentrations in mixed-breed goat bucks receiving testosterone during the non-breeding period. J Appl Anim Res. 43:457–461. doi:10.1080/09712119.2014.980420

Bukar MM, Yusoff R, Haron AW, Goriman Khan, MA, Omar MA. 2012. Estrus response and follicular development in Boer does synchronized with flugeston acetate and PGF2α or their combination with eCG or FSH. Trop Anim Health Prod. 44:1505–1511.

Chemineau P, Daveau A, Maurice F, Delgadillo JA. 1992. Seasonality of estrus and ovulation is not modified by subjecting female Alpine goats to a tropical photoperiod. Small Rumin Res. 8:299–312. doi:10.1016/0921-4488(92)90211-L

Contreras-Villareal V, Meza-Herrera CA, Rivas-Muñoz R, Angel-Garcia O, Luna-Orozco JR, Carrillo E, Mellado M, Véliz-Deras FG. 2016. Reproductive performance of seasonally anovular mixed-bred dairy goats induced to ovulate with a combination of progesterone and eCG or estradiol. Anim Sci J. doi:10.1111/aj.12493. [Epub ahead of print]

Esteves LV, Brandão FZ, Cruz RC, Souza JMG, Oba E, Facó O, Fonseca JF. 2013. Reproductive parameters of dairy goats submitted to estrus synchronization with prostaglandin F2α associated or not to hCG at estrus onset. Arq Bras Med Vet Zootec. 65:1585–1592. doi:10.1590/0950-46452013006000001

FASS. 2010. Guide for the care and use of agricultural animals in agricultural research and teaching. 3rd ed. Champaign, IL: Federation Animal Science Society, 177 p.

Fonseca JF, Bruschi JH, Santos ICC, Viana JHM, Magalhães ACM. 2005a. Induction of estrus in non-lactating dairy goats with different estrus synchrony protocols. Anim Reprod Sci. 85:117–124. doi:10.1016/j.anireprosci.2004.03.005

Fonseca JF, Bruschi JH, Zambrini FN, Demczuk E, Viana JHM, Palhão MP. 2005b. Induction of synchronized estrus in dairy goats with different gonadotrophins. Anim Reprod. 2:50–53.

Fonseca JF, Torres CAA. 2005. Administration of hCG 5 days after breeding and reproductive performance in nulliparous dairy goats. Reprod Dom Anim. 40:495–499.

Ginther OJ, Kot K. 1994. Follicular dynamics during the ovulatory season in goats. Theriogenol. 42:987–1001.

Gonzalez-Bulnes A, Diaz-Delfa C, Garcia-Garcia RM, Urrutia B, Carrizosa JA, Lopez-Sebastian A. 2005. Origin and fate of pre-ovulatory follicles after induced luteolysis at different stages of the luteal phase of the estrous cycle in goats. Anim Reprod Sci. 86:237–245. doi:10.1016/j.anireprosci.2004.07.005

Gonzalez-Bulnes A, Meza-Herrera CA, Rekik M, Ben-Salem H, Kridli RT. 2011. Limiting factors and strategies for improving reproductive outputs of small ruminants reared in semi-arid environments. In: Degenovine, K., editor. Semi-arid environments: agriculture, water supply and vegetation. Hauppauge, NY: Nova Science Publishers Inc.; p. 41–60.

Greggley JPC, Van Niekerk CH. 1990. Effect of pregnant mare serum gonadotrophin (PMSG) and route of administration after progestagen treatment on oestrus and LH secretion in the Boer goat. Small Rumin Res. 3:511–516. doi:10.1016/0921-4488(90)90082-H
Greyling JPC, Van der Nest M. 2000. Synchronization of oestrus in goats: dose effect of progestagen. Small Rumin Res. 36:201–207. doi:10.1016/S0921-4488(99)00165-0

Lehloenya KC, Greyling JPC, Grobler S. 2008. Effect of season on the superovulatory response in Boer goat does. Small Rumin Res. 78:74–79.

López-Sebastian A, González-Bulnes A, Carrizosa JA, Urrutia B, Díaz-Delfa C, Santiago-Moreno J, Gómez-Brunet A. 2007. New estrus synchronization and artificial insemination protocol for goats based on male exposure, progesterone and cloprostenol during the non-breeding season. Theriogenology. 68:1081–1087. doi:10.1016/j.theriogenology.2007.08.003

Luna-Orozco JR, Guillen-Munoz JM, De Santiago-Miramontes MA, Garcia JE, Rodriguez-Martínez R, Meza-Herrera CA, Mellado M, Véliz FG. 2012. Influence of sexually inactive bucks subjected to long photoperiod or testosterone on the induction of estrus in anovulatory goats. Trop Anim Health Prod. 44:71–75. doi:10.1007/s11250-011-9889-y

Meira C, Pessoa VM, Ferreiro JCP, Araujo GHM, Gioso MM, Bicudo SDB, Oba E, Orlandi C. 2006. Alternative low doses and routes of administering a prostaglandin F2α analogue to induce luteolysis in Nelore cows. J Vet Sci. 7:387–390. doi:10.4142/jvs.2006.7.4.387

Mellado J, Veliz F, De Santiago-Miramontes M, Meza-Herrera CA, Mellado M. 2014. Buck-induced estrus in grazing goats during increasing photoperiod and under cold stress at 25° N. Vet Med Zoot. 66:40–45.

Menchaca A, Rubianes E. 2004. New treatments associated with timed artificial insemination in small ruminants. Reprod Fertil Dev. 16:403–413. doi:10.1071/RD04037

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 accreditation of laboratory animal care international. 1st. ed. Harlan.

Russel A, Doney J, Gunn R. 1969. Subjective assessment of body fat in live sheep. J Agric Sci Camb. 72:451–454. doi:10.1017/S0021859600024874

Sarath T, Mehrotra S, Arunmozhi N, Agarwal SK, Hoque M, Mashankar U. 2012. Studies on follicular development and ovarian steroid profile in seasonal anestrus goats. Indian J Anim Reprod. 33:6–8.

Sen U, Onder H. 2016. The effect of estrus synchronization programs on parturition time and some reproductive characteristics of Saanen goats. J Appl Anim Res. 44:376–379. doi:10.1080/09712119.2015.1091348

Simoes J. 2015. Recent advances on synchronization of ovulation in goats, out of season, for a more sustainable production. Asian Pacific J Reprod. 4:157–165. doi:10.1016/S2305-0500(15)30014–2

Simoes J, Almeida JC, Valentim R, Baril G, Azevedo J, Fontes P, Mascarenhas R. 2006. Follicular dynamics in Serrana goats. Anim Reprod Sci. 95:16–26.

Vazquez MI, Blanch MS, Alanis GA, Chaves MA, Gonzalez-Bulnes A. 2010. Effects of treatment with a prostaglandin analogue on developmental dynamics and functionality of induced corpora lutea in goats. Anim Reprod Sci. 118:42–47. doi:10.1016/j.anireprosci.2009.05.016

Vilariño M, Rubianes R, 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.

Viñoles C, Meikle A, Forsberg M, Rubianes E. 1999. The effect of subluteal levels of exogenous progesterone on follicular dynamics and endocrine patterns during the early luteal phase of the ewe. Theriogenology. 51:1351–1361.