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Serum Progesterone Profiles in Saanen Crossbred Goats During a 5-day Progestin-Based Estrous Synchronization Protocol

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ABSTRACT

A short-term progestin-based estrous synchronization protocol was tested using a 5-day progesterone plus a luteolytic dose of prostaglandin (PG) and equine chorionic gonadotropin (eCG) in Saanen crossbred dairy goats. Synchronization was monitored by measuring serum progesterone (P4) via enzyme immunoassay. The treatment was initiated by inserting an intravaginal progesterone release device (CIDR-G) at day 0 (D0) along with intramuscular cloprostenol 125 μg, followed by 100 IU eCG on D3. The CIDR-G was removed on D5. After treatment, estrus was observed twice daily using a vasectomized buck. Blood samples were collected daily from D0 until D11 for serum P4 analysis. Eight out of nine (89%) does came into estrus with an average onset of estrus at 45.0 ± 4.4 hr (mean ± SE) after CIDR-G removal. The P4 concentration immediately before the treatment was 22.4 ± 5.3 ng.mL^{-1} (range 1.3 to 37.8 ng.mL^{-1}). The highest concentration appeared on D1 and the lowest on D7. There was a difference between serum P4 concentrations during CIDR-G insertion (D1–D5) and after CIDR-G removal (D6–D9) (26.0 ± 2.4 and 3.4 ± 0.4 ng.mL^{-1}, respectively, $P < 0.001$). The P4 profiles implied that this protocol based on 5-day progestin-based estrous synchronization can be applied successfully to crossbred dairy goats.

Keywords: goat, progestin-based estrous synchronization, progesterone, equine chorionic gonadotropin (eCG), $PGF_{2\alpha}$

INTRODUCTION

The purpose of estrous synchronization is to bring a whole group of animals into estrus and to be naturally or artificially inseminated at the same time. In goats, a classical scheme for estrous synchronization involves long-term progesterone treatment, long enough for corpus lutea to undergo regression in all the animals irrespective of the cycle status of each animal at the beginning of the treatment (Holtz, 2005). The most widely used method uses progesterone or progestagen for 9–11 d followed by a luteolytic dose of prostaglandin (PG), or an analog, administered 48 hr prior to the end of the treatment (Baldassarre and Karatzas, 2004; Holtz, 2005; Fatet *et al.*, 2011). Equine chorionic gonadotropin (eCG) supplement has been routinely used, particularly for out-of-season breeding, either at the time of progesterone withdrawal or 1–2 d before. This protocol promotes follicle development, initiates the preovulatory events and synchronizes ovulation thus allowing artificial insemination (AI) to be performed at predetermined times

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(Wheaton *et al*., 1993; Wildeus, 2000; Baldassarre and Karatzas, 2004; Holtz, 2005; Abecia *et al.*, 2012). Generally, doses of 200 to 600 IU of eCG should be adjusted for each animal according to the season, breed, weight, age, desire for multiple kidding and previous responses (Baldassarre and Karatzas, 2004; Holtz, 2005; Fatet *et al*., 2011).

The follicular wave in goat emerges every 5–7 d (Rubianes and Menchaca, 2003) or 4–6 d (Cruz *et al*., 2005). The dominant follicle of any follicular wave, can ovulate if the appropriate endocrine conditions are provided, that is, by injection of prostaglandin $F_{2\alpha}$ ($PGF_{2\alpha}$) to induce luteolysis (Fortune *et al*., 2001). New strategies based on the current understanding of follicular dynamics and manipulation of preovulatory follicular development using hormonal control could offer shorter protocols of 4–7 d. Despite this knowledge, the traditional long-term (11–17 d) progesterone treatment has been common practice (Menchaca *et al*., 2007).

In tropical climates, goats continue their reproductive cyclicity throughout the year (Chemineau *et al*., 2004; Fatet *et al*., 2011). However, the incidence of aberrant short estrous cycles, as short as 4 d, caused by premature regression of corpus lutea has been reported (Eiamvitayakorn *et al*., 1988; Cerbito *et al*., 1995). Eiamvittayakorn *et al.* (1988) documented the normal ovulation rates after short estrous cycles and concluded that the occurrence of short estrous cycles was a normal phenomenon in goats that provided a good opportunity for rebreeding within short intervals. Nevertheless, such erratic cycling impacts the breeding management. Thus, short-term synchronization protocols may help overcome these variables which otherwise impinge on the reproductive cycle of the tropical goat. The present study, therefore, sought to test the efficacy of a 5-day progestin-based estrous synchronization protocol in Saanen crossbred dairy goats using serum progesterone (P4) to monitor the synchronization effect.

MATERIALS AND METHODS

This study was conducted in Chiang Mai province, Thailand during April 2011 (average daily temperature range 23–36 °C). Nine healthy, non-pregnant and non-lactating Saanen crossbred does, aged between 2 and 5 yr, body condition score $\geq$ 2.5 (ranging from 1 to 5), average body weight 43.8 kg (range 32–54 kg) were used for the experiment. All does were fed with 18% protein concentrate, 0.5 kg per doe per day. They were allowed to graze on pastures during the day and housed in pens at night. Mineral salts and water were provided *ad libitum*. The protocol for animal use in this experiment was approved by the Institutional Animal Care and Use Committee, Faculty of Veterinary Medicine, Chiang Mai University.

A 5-day progestin-based estrous synchronization protocol (Figure 1) was used. The controlled internal drug release device, CIDR-G (EAZI-Breed™ CIDR®, Pfizer, Auckland, New Zealand) containing 0.3 g progesterone was used as the intravaginal progesterone delivery system. Day 0 (D0) was designated as the day of CIDR-G insertion and concurrently 125 µg cloprostenol (EstroPLAN®, Parnell Laboratories (AUST) Pty. Ltd., Alexandria, NSW, Australia) was administered intramuscularly. One hundred IU of eCG (Folligon®, Intervet International, Boxmeert, the Netherlands) was intramuscularly injected 2 d (D3) before the withdrawal of the CIDR-G.

Following the 5-day treatment, estrus was detected at 12-hour intervals by observation of behavioural changes with a vasectomized buck. Onset of estrus was defined as the moment when a doe stood to be mounted by a buck. Transcervical artificial insemination was done using frozen semen 54 hr after CIDR-G removal. Pregnancy diagnosis was performed by transrectal ultrasonography using a 5 MHz probe at 70 d after breeding.

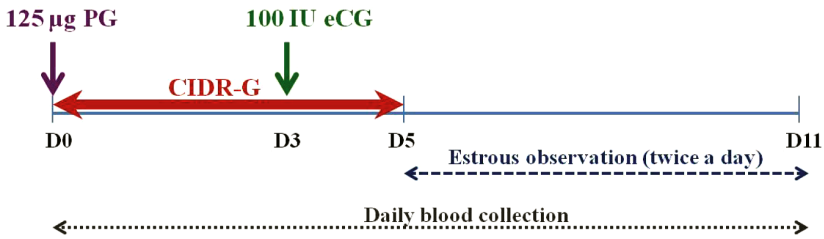

Figure 1 Short-term estrous synchronization in goats using the intravaginal progesterone release device (CIDR-G)-based protocol in combination with prostaglandin (PG) on day 0 and equine chorionic gonadotropin (eCG) on day 3.

From D0 to D11, 5 mL blood samples were taken daily via jugular venepuncture, centrifuged at 1500× *g* for 15 min and the serum stored at −20 °C for later progesterone (P4) analysis. These were measured by enzyme immunoassay (EIA), as described by Brown, (2008), using a monoclonal antibody (CL 425) courtesy of Dr. Coralie Munro (University of California, Davis, CA, USA). An ELISA Reader (SUNRISE® Absorbance reader, Tecan Austria GmbH, Salzburg, Austria) was used to measure the optical density of each well at 405 nm. The sensitivity of the assay was 0.04 ng.mL^{-1} of serum. The intra- and inter-assay coefficients of variation were less than 10% and less than 15%, respectively.

The onset of estrus and estrous duration were recorded. All data were presented as the mean ± SE. The differences between groups were statistically tested with Student's *t*-test and were considered to be significant at the $P < 0.05$ level.

RESULTS

Following the treatment, eight out of nine (89%) does showed clear signs of estrus with an average onset of estrus after CIDR-G removal occurring at 45.0 ± 4.4 hr (range 36–72 hr). For the does that showed sign of estrus, the mean duration of estrus was 28.5 ± 3.9 hr (range 12–48 hr). The distribution of estrous behaviour was within 1.5 d with 7 out of 8 does expressing signs of estrus 36–48 hr after CIDR-G withdrawal. A delayed estrus was observed in one goat at 72 hr.

The average serum P4 concentration on the first day (D0) before the treatment started was 22.4 ± 5.3 ng.mL^{-1} (ranging from 1.3 to 37.8 ng.mL^{-1}). The profile peaked on D1 at the level of 31.9 ± 2.3 ng.mL^{-1} and fell to the lowest concentration on D7 at 2.5 ± 0.3 ng.mL^{-1}.

Serum P4 profiles monitored throughout the study are shown in Figure 2. The concentrations were at the highest level (31.9 ± 2.3 ng.mL^{-1}) on the following day (D1) after the insertion of CIDR-G, and then slightly declined during the treatment period. After withdrawal of the progesterone release device, the level dropped abruptly to the lowest concentrations (2.5 ± 0.3 ng.mL^{-1}) at D7, remained at a low level until D9 and then increased to 8.0 ± 1.5 ng.mL^{-1} on the last day of P4 monitoring.

There was a clear difference between the serum P4 concentrations during CIDR-G insertion (D1–D5) and after the device removal (D6–D9); the average of D1–5 was 26.0 ± 2.4 ng.mL^{-1} falling to 3.4 ± 0.4 ng.mL^{-1} on D6–D9, ($P< 0.001$). The progesterone concentrations on D11 elevated from the level of 3.4 ± 0.4 ng.mL^{-1} (D6–D9) to 8.0 ± 1.5 ng.mL^{-1} ($P < 0.001$). Of the nine does, six had high P4 serum concentrations at the beginning of the trial, ranging from 24.3 to 37.8 ng.mL^{-1} and the remaining three does had lower concentrations, ranging from 1.3 to 2.3 ng.mL^{-1}. The non-estrus doe showed high serum P4 during the 5-day treatment; the mean P4 concentration was 21.3

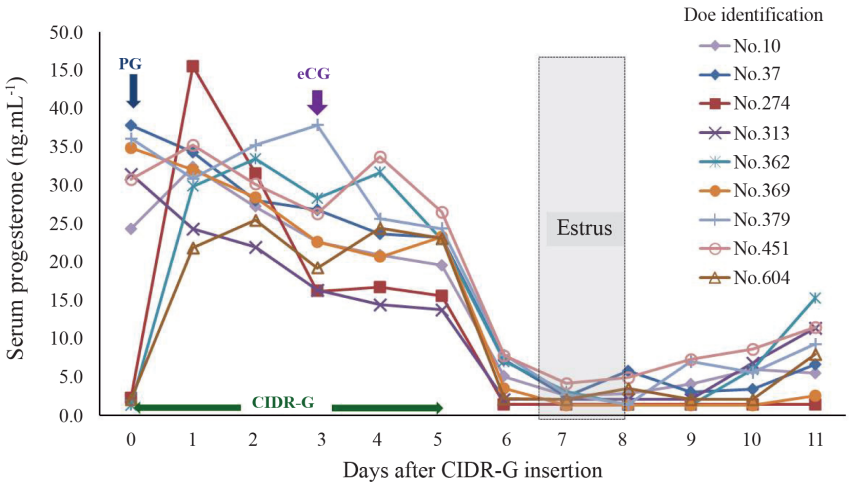

Figure 2 Serum progesterone concentrations of does (n = 9) from the start of the progestin-based estrous synchronization protocol. (PG = prostaglandin; eCG = equine chorionic gonadotropin; CIDR-G = intravaginal progesterone release device.)

± 5.0 ng.mL^{-1}, ranging 2.3 (D0) to 45.5 ng.mL^{-1} (D1); but from D6 to D11, P4 remained constant at the base line level (1.5 ng.mL^{-1}).

A single transcervical insemination was performed 54 hr after CIDR-G withdrawal and four of the eight does (50%) were pregnant as confirmed by ultrasongraphy at 70 d of gestation.

DISCUSSION

From the P4 profiles, the wide variation in serum P4 at D0 (Figure 2) indicated different stages of reproduction between does. Before starting the synchronization protocol, P4 serum concentrations in six of the nine does were high, ranging from 24.3 to 37.8 ng.mL^{-1}, indicating that they were in the luteal phase. The other three does with baseline concentrations (1.3, 2.1 and 2.3 ng.mL^{-1}) were probably in the follicular phase of the cycle. One of these three (number 274) did not show estrus and had a persistently low P4 concentration (1.5 ng.mL^{-1}) after CIDR-G withdrawal until D11 which suggests that the animal was not cycling. This doe also had the lowest weight in the group (32 kg). Artificial insemination was not performed on this doe.

In this study, prostaglandin given at the beginning of the treatment caused luteolysis of the corpus lutea (if any), hence serum P4 concentrations from D1 to D5 were from the exogenous progesterone. After CIDR-G insertion, the P4 concentrations were uniformly high during the first day irrespective of the starting levels at D0 and decreased immediately after the device withdrawal. These findings accord with similar serum hormone profiles obtained with CIDR-G treatment in goats (Menchaca *et al*., 2007; Rowe *et al*., 2010; Souza *et al*., 2011; Vilarino *et al*., 2011).

The understanding of goat ovarian dynamics has led to new strategies in controlling follicular development using short exposures to progestagen within the time frame of the follicular wave interval (4–7 d). The present findings can be explained by the progesterone derived from the CIDR-G causing inhibited ovulation of the largest follicles of both anovulatory and ovulatory waves. The lutenizing hormone (LH) pulse frequency presumably increased after a synchronous

decrease in circulating progesterone at the device withdrawal, allowing LH-dependent follicles to grow to pre-ovulatory size and eventually ovulate. Administration of eCG on D3, on the other hand, induced final growth of dominant follicles, allowing synchronous ovulation. However, in cows, progestin exposures exceeding the lifespan of a dominant follicle can lead to the development of a persistent follicle containing an aged oocyte and hence deteriorating fertility (Mihm *et al*., 1999). Thus even a 1.5 d delay compromised embryo quality, although fertility was maintained (Cerri *et al*., 2009). Likewise, a study in sheep showed that extending the lifespan of follicles from the penultimate wave by using a single dose of $PGF_{2\alpha}$ and a 6-day intravaginal progesterone sponge reduced functional viability leading to atresia of follicles containing oocytes of poor quality (Seekallu *et al*., 2010).

Various forms of progesterone or progestagens have long been used for goat estrous synchronization both out-of-season and during the breeding season, with or without prostaglandin or gonadotropins (Whitley and Jackson, 2004). Menchaca and Rubianes (2004) suggested that it was necessary to associate the short treatment with eCG using either 200 or 400 IU when the progesterone device is removed to gain the acceptable estrous response (Menchaca and Rubianes, 2004). In the present study, a lower dose of 100 IU eCG was administered 2 d before CIDR-G removal which resulted in a more delayed interval from the end of treatment to the onset of estrus (45.0 ± 4.4 hr) compared to 30 hr in an earlier report (Menchaca and Rubianes, 2004). In a previous report on ewes (Ali, 2007), the eCG given 2 d before progestin withdrawal produced a more synchronised estrus and ovulation compared to these procedures being performed at the same time. Indeed, the present results imply that such a protocol can create a coordinated estrus with a tight distribution of estrous behaviour within 1.5 d, with seven out of eight does expressing signs of estrus 36–48 hr after CIDR-G withdrawal.

The key features of successful estrous synchronization are to obtain close synchronization, a rapid decline in circulating progesterone, and co-ordinated growth and ovulation of viable follicles (Mapletoft *et al*., 2003). This has been confirmed in goats using a short-term synchronization protocol during the nonbreeding season (Vilarino *et al*., 2011) where 100% ovulation was observed by ultrasonography with ovulation occurring between 60–70 hr after CIDR-G removal and achieving pregnancy rates after AI of 75%. Although in the present study there was no direct observation of ovulations, the raised serum progesterone concentration on D11 (6 d after CIDR-G removal) was significantly higher than during the follicular phase (D6–D9) in all eight goats that showed estrous expression indicating that ovulation had occurred thus permitting successful fertilization. In the present study, four out of eight (50%) does conceived after transcervical artificial insemination using frozen semen at 54 hr after CIDR-G removal.

The short-term use of CIDR-G may help reduce some undesirable effects reviewed as follows: (i) foul-smelling discharges (Wheaton *et al*., 1993), (ii) discomfort and adhesion of the device to the vagina wall (Holtz, 2005), (iii) vaginitis (Lopez-Sebastian *et al*., 2007 Abecia *et al*., 2011) which can be associated with long vaginal retention times. Furthermore, food safety issues arising from hormone residues in milk will be reduced with the short-term protocol, thus benefitting dairy farmers.

In conclusion, these experiments demonstrated the effectiveness of a 5-day progesterone-based protocol for estrous synchronization in Saanen crossbred goats in a tropical environment where erratic cycles have been reported. The protocol can be applied at any stage of the cycle. The number of animals tested was small, so that data can only be considered as preliminary. Further study on determination of the LH peak and the ovulation time after the end of this proposed short-term protocol using CIDR-G

combined with 125 µg cloprostenol and 100 IU eCG should be undertaken so that timed AI can be 'fine-tuned'.

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LITERATURE CITED

Abecia, J.A., F. Forcada and A. Gonzalez-Bulnes. 2011. Pharmaceutical control of reproduction in sheep and goats. **Vet. Clin. North Am. Food Anim. Pract.** 27: 67–79.

Abecia, J.A., F. Forcada and A. Gonzalez-Bulnes. 2012. Hormonal control of reproduction in small ruminants. **Anim. Reprod. Sci.** 130: 173–179.

Ali, A., 2007. Effect of time of eCG administration on follicular response and reproductive performance of FGA-treated Ossimi ewes. **Small Ruminant Research** 72: 33–37.

Baldassarre, H. and C.N. Karatzas. 2004. Advanced assisted reproduction technologies (ART) in goats. **Anim. Reprod. Sci.** 82-83: 255–266.

Cerbito, W.A., N.G. Natural, F.B. Aglibut and K. Sato. 1995. Evidence of ovulation in goats (*Capra hircus*) with short estrous cycle and its occurrence in the tropics. **Theriogenology** 43: 803–812.

Cerri, R.L.A., H.M. Rutigliano, R.C. Chebel and J.E.P. Santos. 2009. Period of dominance of the ovulatory follicle influences embryo quality in lactating dairy cows. **Reproduction** 137: 813–823.

Chemineau, P., A. Daveau, Y. Cognie, G. Aumont and D. Chesneau. 2004. Seasonal ovulatory activity exists in tropical creole female goats and black belly ewes subjected to a temperate photoperiod. **BMC Physiol.** 4: 12.

Cruz, J.F., D. Rondina and V.J.F. Freitas. 2005. Ovarian follicular dynamics during anoestrus in Anglo-nubian and Saanen goats raised in tropical climate. **Trop. Anim. Health Prod.** 37: 395–402.

Eiamvitayakorn, J., E.M. Rigor, B.R. Garcia and C.L. Apelo. 1988. Aberrant estrous cycles in goats. *In* **Proceedings of 11th International Congress on Animal Reproduction and Artificial Insemination**, Dublin, Ireland. pp. 22–24.

Fatet, A., M.-T. Pellicer-Rubio and B. Leboeuf. 2011. Reproductive cycle of goats. **Anim. Reprod. Sci.** 124: 211–219.

Fortune, J.E., G.M. Rivera, A.C.O. Evans and A.M. Turzillo. 2001. Differentiation of dominant versus subordinate follicles in cattle. **Biol. Reprod.** 65: 648–654.

Holtz, W. 2005. Recent developments in assisted reproduction in goats. **Small Rumin. Res.** 60: 95–110.

Lopez-Sebastian, A., A. Gonzalez-Bulnes, J.A. Carrizosa, B. Urrutia, C. Diaz-Delfa, J. Santiago-Moreno and A. Gomez-Brunet. 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.

Mapletoft, R.J., M.F. Martinez, M.G. Colazo and J.P. Kastelic. 2003. The use of controlled internal drug release devices for the regulation of bovine reproduction**. J. Anim. Sci.** 81: E28–36.

Menchaca, A., V. Miller, V. Salveraglio and E. Rubianes. 2007. Endocrine, luteal and follicular responses after the use of the short-term protocol to synchronize ovulation in goats. **Anim. Reprod. Sci.** 102: 76–87.

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

Mihm, M., N. Curran, P. Hyttel, P.G. Knight, M.P. Boland and J.F. Roche. 1999. Effect of dominant follicle persistence on follicular fluid oestradiol and inhibin and on oocyte maturation in heifers. **J. Reprod. Fertil.** 116: 293–304.

Rowe, J.D., L.A. Tell, J.L. Carlson, R.W. Griffith, K. Lee, H. Kieu, S. Wetzlich and D. Hallford. 2010. Progesterone milk residues in goats treated with CIDR-G® inserts. **J. Vet. Pharmacol. Ther.** 33: 605–609.

Rubianes, E. and A. Menchaca. 2003. The pattern and manipulation of ovarian follicular growth in goats. **Anim. Reprod. Sci.** 78: 271–287.

Seekallu, S.V., B.M. Toosi, A.T. Grazul-Bilska and N.C. Rawlings. 2010. Markers of ovarian antral follicular development in sheep: Comparison of follicles destined to ovulate from the final or penultimate follicular wave of the estrous cycle. **Reproduction** 140: 559–568.

Souza, J.M.G., C.A.A. Torres, A.L.R.S. Maia, F.Z. Brandao, J.H. Bruschi, J.H.M. Viana, E. Oba and J.F. Fonseca. 2011. Autoclaved, previously used intravaginal progesterone devices induces estrus and ovulation in anestrous Toggenburg goats. **Anim. Reprod. Sci.** 129: 50–55.

Vilarino, M., E. Rubianes and A. Menchaca. 2011. Re-use of intravaginal progesterone devices associated with the short-term protocol for timed artificial insemination in goats. **Theriogenology** 75: 1195–1200.

Wheaton, J.E., K.M. Carlson, H.F. Windels and L.J. Johnston. 1993. CIDR: A new progesterone-releasing intravaginal device for induction of estrus and cycle control in sheep and goats. **Anim. Reprod. Sci.** 33: 127–141.

Whitley, N.C. and D.J. Jackson. 2004. An update on estrus synchronization in goats: A minor species. **J. Anim Sci.** 82: E270–276.

Wildeus, S. 2000. Current concepts in synchronization of estrus: Sheep and goats. **J. Anim. Sci.** 77: 1–14.