Effect of Short-term and Long-term Synthetic Progesterone on Estrous Synchronization and Conception Rate in Thai-native Goat

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ABSTRACT

The aim of the study was to compare the efficiency of short-term and long-term synthetic progesterone on estrous synchronization and conception rate in Thai-native goat. Two experiments were conducted to meet the objectives of the research as follows. Experiments 1, Thai-native goats (8-10 months of age) were randomly from the herd with the average weight of 16.9 kg. The goats were randomly assigned into two groups different treatment as follow: Treatment 1 goats received intravaginally controlled internal drug release (CIDR) device 0.3 g of progesterone for 14 days (long-term protocol) with the combination 0.5 ml $PGF_{2\alpha}$ and 150 IU PMSG or Treatment 2 goats received intravaginally CIDR for 5 days (short-term protocol) with the same combination of $PGF_{2\alpha}$ and PMSG. Plasma progesterone concentrations after CIDR removal at 0-96 h in goats received long-term protocol significantly lower than goats received short-term protocol indicating that goats responded for long-term protocol than short-term protocol ($P<0.05$). In experiments 2, long-term protocol was choosen for estrous synchronization and artificial insemination in crossbred goats. Animals (n=16), 8-10 months of age, were estrous synchronized using CIDR (14 days), $PGF_{2\alpha}$, and PMSG. Cervical artificial insemination was performed using frozen semen at 54 hours after CIDR removed. Blood samples were taken on 80 days post AI to confirm pregnancy status. Two out of sixteen goats were determined pregnancy (12.5%) according to high progesterone concentrations (>5 ng/ml), whereas the fourteen goats remain in doubt. Despite high progesterone concentrations after AI, the conception rate in goats was still low, probably due to the quality of frozen semen and AI technique.

Key words: Conception rate, Estrous synchronization, Synthetic progesterone, Thai-native goat

INTRODUCTION

Thai-native goat is one of potential livestocks to be developed. However, difficulties on reproductive management, i.e., estrous detection, and unknown time of ovulation, cause low reproduction performance of goats. Estrous synchronization is a key element of all the assisted reproductive technologies (ARTs) protocols in livestock animals and has a major influence to increase the overall efficiencies of reproduction (Baldassarre and Karatzas, 2004). Estrous synchronization plays a major role in fixed time breeding. The value of estrous synchronization is vital in goats as the duration of both estrous cycle and estrus is variable and estrous detection cannot be accomplished safely without a buck (Rahman et al., 2008).

The most widely used procedures for estrous synchronization and induction of estrus in small ruminants are a combination of an intravaginal devices impregnated with 0.3 g of progesterone (controlled internal drug release, CIDR) with an intramuscular injection of PMSG and $PGF_{2\alpha}$. An alternative means of supplying continuous, exogenous progesterone has been the CIDR developed for goats in New Zealand. The CIDR device is constructed from natural progesterone impregnated

medical silicone elastomer molded over a nylon core. CIDR contain low natural doses of progesterone (Wheaton et al., 1993), currently the CIDR and subcutaneous implants are preferable than sponges because these are easy to use (Holtz, 2005), and CIDR do not absorb or obstruct drainage of vaginal secretions, resulting in less foul-smelling discharge upon removal (Motlomelo et al., 2002; Romano, 2004). Ovulation can be synchronized more precisely by administering GnRH around the time of estrus (Pierson et al., 2003), which improves the success of fixed-time artificial insemination.

Therefore, the objective of the present study was to compare the efficiency of short-term and long-term synthetic progesterone on estrous synchronization, plasma progesterone concentrations, and conception rate in Thai-native goat.

MATERIALS AND METHODS

The experiment was approved by the Animal Ethics Committee of Khon Kaen University (Reference No. 0514.1.12.2/67; January 13th, 2011).

Two experiments were carried out at the small ruminant unit, Department of Animal Science, Faculty of Agriculture, Khon Kaen University, located at 16° 26′ N latitude and 102° 50′ E longitude, Thailand. Animals were fed with a maintenance diet (NRC, 1981) and *ad libitum* feeding of fresh ruzi grass. Clean water and mineral block were provided for all animals. Animals were vaccinated against foot and mouth disease (FMD) and brucellosis, according to the standard farm requirement of the Department of Livestock Development, Ministry of Agriculture and Cooperatives, Thailand.

Experiment 1 Comparison the efficiency of short-term and long-term synthetic progesterone on plasma progesterone concentrations in Thai-native goat

Animals and treatments

Twelve nulliparous Thai-native female goats, 8-10 months of age, the females with a body condition score of 2.5-3.0 and body weight of 16.9 kg, were randomly allocated to two treatment groups using a completely randomized design. Induction of estrus and plasma progesterone concentrations of goats in each of the groups were synchronized with one of the following treatment. Treatment 1, long-term protocol (n=6) using CIDR+$PGF_{2\alpha}$ and PMSG: Animal were randomly treated with intravaginal CIDR 0.3 g progesterone (Eazi-BreedTMCIDR®, Pfizer, NY, USA) for 14 d (Day 0-14), i.m. injection of $PGF_{2\alpha}$ (Estrumate® 0.5 ml) and PMSG (Folligon®) 150 IU (0.75 ml) were given at withdrawal (Day 14). Treatment 2, short-term (n=6) using CIDR+ $PGF_{2\alpha}$ and PMSG: Animal were randomly treated with intravaginal CIDR 0.3 g progesterone (Eazi-BreedTMCIDR®, Pfizer, NY, USA) for 5 d (Day 0-5), and i.m. injection of $PGF_{2\alpha}$ (Estrumate® 0.5 ml) and PMSG (Folligon®) 150 IU (0.75 ml) were given at withdrawal (Day 5).

Enzyme-linked immunosorbant assay (ELISA)

A blood sample (5 ml) for progesterone analysis was collected via jugular venipuncture from Day-1 to 5 with higher frequency at device insertion (Day 0, Day 0+4 h, D 0+12 h) and after CIDR removal at 0-96 h follow by short-term protocol and Day -1 to 14 with higher frequency at device insertion (Day 0, Day 0+4 h, D 0+12 h) and after CIDR removal at 0-96 h follow by long-term protocol into an EDTA solution, then immediately centrifuged at 1500 × g for 15 min. Blood plasma samples were harvested and frozen stored at -20°C until assayed. Progesterone concentrations were determined by Enzyme-linked immunosorbant assay or ELISA (Cushwa et al., 1992). The intra-assay coefficient of variation was 5.3%, and assay sensitivity was 0.025 ng/ml.

Ovarian ultrasonography

Ovarian follicular dynamics were monitored by transrectal ultrasonography using a 7.5 MHz transducer (HS-2000, HONDA ELECTRONICS, Japan) stiffened with a hollow plastic rod. Ovarian

ultrasonography was performed by the same operator once a day from 2 d before device insertion to device removal, twice a day from 2 d after device removal (at 24 and 48 h), and once a day until ovulation (at 72 and 96 h after device removal).

Estrous detection

Estrus was detection performed every 8 h for 96 h after device withdrawal. The onset of the estrus was recorded when the female exhibited standing heat by the vasectomized buck.

Experiment 2 Effect of long-term progesterone in fixed time AI on pregnancy rate in Thai-native goat

Sixteen nulliparous Thai-native goats, 8-10 months and body weight of 16.9 kg. The goats received the same long-term protocol used in experiment 1, consisting of 14 d of progesterone treatment with a CIDR. One i.m. dose of 0.5 ml $PGF_{2\alpha}$ and 150 IU PMSG was given at withdrawal, respectively. Fixed-time cervically artificial insemination of frozen semen (200×10^6 spermatozoa per dose) was performed 54 h after CIDR withdrawal. Pregnancy rate (pregnancy goats/insemination goats) was determined 30-35 d after insemination by transrectal ultrasound (7.5 MHz, HS-2000, HONDA ELECTRONICS, Japan) and blood samples were taken on 80 days after AI to confirm pregnancy rate.

Statistical analyses

Data were expressed as means±SEM and compared by ANOVA using GLM procedure of SAS (2001). Conception rate were determined using the concentration of progesterone and compared by a Student *t*-test. Means were considered significantly different if $P<0.05$.

RESULTS

Experiment 1

The results showed there was statistical difference between the treatment groups in estrous response ($P<0.05$), long-term protocol allows an acceptable estrous response, it was greater than short-term protocol. Similarly plasma progesterone concentrations after CIDR removal at 0-96 h in goats received long-term protocol significantly ($P<0.05$) lower than goats received short-term protocol (Figure 1).

Average plasma progesterone concentrations was 5.0±0.4 ng/ml during insertion CIDR of long-term protocol similar with 5.3±0.4 ng/ml of short-term protocol immediately before CIDR insertion (2.3±1.6 and 2.7±0.4 ng/ml, respectively). These data indicate that progesterone from the CIDR is readily absorbed through the vagina and rapidly enters the circulation. All CIDRs of long-term protocol were removed on Day 14. Immediately CIDR removal, plasma progesterone averaged 5.8±3.1 ng/ml and plasma progesterone concentrations continued to decline from the average to 1.5±0.8, 0.6±0.3, and 0.8±0.4 ng/ml at 24, 48, and 72 h after CIDR removal, respectively. Whereas in short-term protocol, plasma progesterone concentrations decline less than long-term protocol at 24, 48, and 72 h after CIDR removal (4.0±0.2, 2.9±0.2, and 2.7±0.3 ng/ml, respectively).

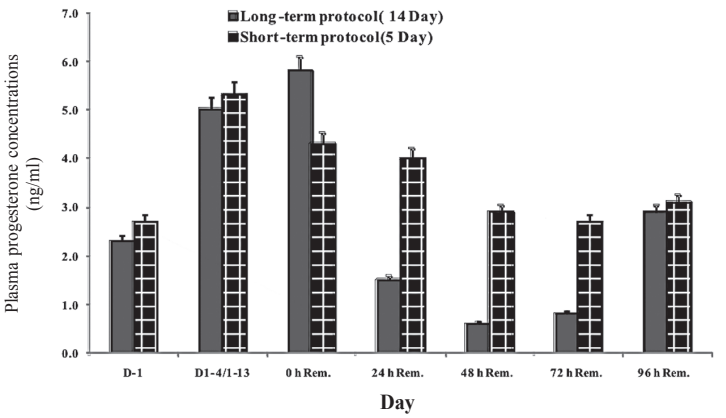

Figure 1. Plasma progesterone concentration in Thai-native goat receiving long-term (14 Day) and short-term protocols (5 Day).

Experiment 2

Plasma were progesterone concentrations after hormone removal, fourteen goats (14/16) had on day 80 after AI were >1 ng/ml (average 3.87 ng/ml), progesterone concentrations between 1-5 ng/ml and two out of sixteen goats were determined as pregnant animal (12.5%) according to high progesterone concentrations (>5 ng/ml), whereas the remains were certainly not pregnant. Despite high progesterone concentrations after AI, the conception rate in goats was still low, probably due to the quality of frozen semen and AI technique (Figure 2).

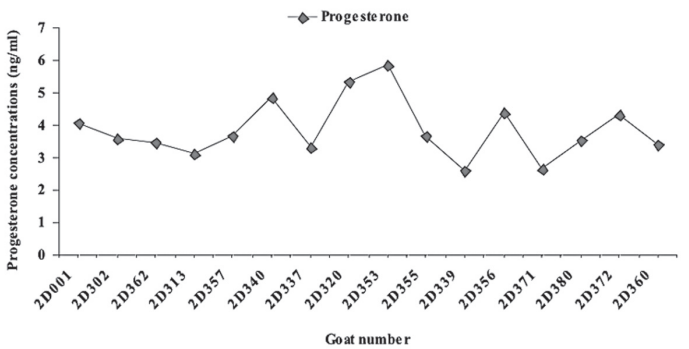

Figure 2. Plasma progesterone concentrations on day 80 after AI of Thai-native goats.

DISCUSSION AND CONCLUSION

In the present study, we demonstrated that the plasma progesterone concentrations after CIDR removal at 0-96 h in goats received long-term protocol significantly was lower than goats received short-term protocol indicating that goats responded for long-term protocol more than short-term protocol and the estrous response after CIDR removal in long-term was significantly higher than in short-term. Long-term protocol (14 d) with $PGF_{2\alpha}$ and PMSG affected the efficiency of the estrous response and plasma progesterone concentrations in Thai-native goats. This is in agreement with Romano (2004), Dixon et al. (2006), and Motlomelo et al. (2002), who reported 97-100% of animals in estrus after 13, 12, and 16 d protocols with CIDR, respectively, using $PGF_{2\alpha}$ and PMSG. This study demonstrated that the length of synthetic progesterone treatment affected estrous response. Seventy-two hours after CIDR removal, plasma progesterone of long-term protocol declined from

5.8±0.4 to 0.8±0.4 ng/ml at CIDR removal and declined more than short-term protocol (2.7±0.3 ng/ml at 72 h after CIDR removal). These data indicate that long-term protocol was more effective than short-term protocol in term of estrus induction in Thai-native goat. One possible explanation for this fact reduced levels of plasma progesterone were not enough to suppress the hypothalamic-pituitary axis, resulting in the estrous response (Romano, 2004; Ungerfeld and Rubianes, 2002).

However, the use of long-term protocols results in a good synchronization of estrus but conception rate is generally depressed probably due to the quality of frozen semen and AI technique. This is in agreement with Donovan et al. (2004) who reported that cervical insemination with frozen-thawed semen also gives low fertility. Kinder et al. (1996) and Rubianes and Menchaca (2003) reported that long-term progestin treatment induces the growth of persistent follicles lowering infertility of goats. Long-term progestin treatment exhibited detrimental effects to the oocyte because the oocyte was too old resulting in decreased fertility (Kinder et al., 1996).

The efficacy of synchronization of estrous depends on many factors, including estrous synchronization technique, body condition, age of puberty, time and method of insemination, location (vagina, cervix, trancervical or uterus) of insemination, quality and quantity of semen inseminated (number of live sperm cells), and semen (fresh or frozen) handling for AI, (Baldassare and Karatzas, 2004; Whitley and Jackson, 2004). For examples, Mellado et al. (2004) reported that conception rates of goats having BCS 3 were around 20% lower than rates of goats having BCS 4 or greater. Meza-Herrera et al. (2008) documented that does with higher BCS have more CL than does with lower condition. Additionally, Serin et al. (2010) demonstrated that a low BCS (<1.5) at the start of the AI programs period caused the low conception rate. These breeds reach puberty at an age of 7-12 months in temperate countries, and become sexually competent at an age 12-20 months in the tropics. Freitas et al. (2004) indicated that mean body weight at puberty was 26.4 kg for Anglo-Nubian and 22.5 kg for Saanen goats, but are more when compared with the mean body weight in the present study has been set at 16.9 kg for Thai-native goats (Lertchunhakiat et al., 2008). The relationship between the early onset of estrus, ovulation and pregnancy rates after AI programs, Baril et al. (1993) and Romano (2004) reports showed that when goats exhibited estrus until 30 h after progestagen treatment, a fertility rate of 65% was observed following AI using frozen-thawed semen, but when estrus was observed at intervals of 49 or 72 h after hormone treatment, a pregnant goat rate of only 25% was verified (Baril et al., 1993). For this reason, some researchers such as Nogueira et al. (2011) demonstrated that the mean fertility and prolificacy after AI using fresh extended semen was considered excellent compared to the frozen semen.

In conclusion, the use synthetic progesterone (CIDR) on estrous synchronization in long-term protocol was more effective than short-term protocol to induce estrus in Thai-native goat. Despite high progesterone concentrations after artificial insemination, the conception rate in goats was still low, probably due to the quality of frozen semen and artificial insemination technique as described herein.

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