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Original article

Efficiency comparison of first use and re-use synthetic progesterone on estrus synchronization and pregnancy rates after natural breeding and Timed AI in goats

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Abstract The aim of the study was to compare the efficiency of first use and re-use synthetic progesterone on estrus synchronization and pregnancy rate after natural breeding and Timed artificial insemination (TAI) in goats. Experiment 1, goats (n=28) with the average age and body weight of 10 months and 17 kg received 14 days of controlled internal drug release (CIDR) device (the first use; Eazi-Breed™CIDR®) or the re-use of CIDR device. All goats were given 300 IU injection of hCG (Chorulon®) at the day of CIDR withdrawal. No differences in percentage of estrus and ovulation were observed. However goats, received the re-use of CIDR device, exhibited shorter (P<0.05) duration of estrus (21.3 h) compared to goats in the first use (27.0 h) and delayed estrus and time of ovulation (70.8 and 93.3 h) compared to the first use of CIDR (38.5 and 67.0 h). Plasma progesterone (P4) concentrations were significantly different (P<0.05) between treatments during CIDR device insertion and after CIDR withdrawal. Experiment 2, goats (n=52) were estrus synchronized using the first use of 14 days CIDR, and were randomly assigned into 2 treatments. Treatment 1, goats (n=16) were naturally bred with the mature male breeder at 12 h after the onset estrus. Treatment 2, goats (n=36) were cervically TAI at 56 h after CIDR withdrawal with frozen semen (200×10^6 spermatozoa per dose). Pregnancy rate was determined 70 day after breeding by transrectal ultrasonography and P4 concentrations. Pregnancy rates were 100% and 22.2% (P<0.05) for natural breeding and TAI. This study indicates that estrus synchronization protocols in goat are not different between those two protocols. In addition, the re-use synthetic progesterone could be used in goat to synchronize the estrus. Although TAI could be used in goat, pregnancy rate was lower campared to the natural breeding. Therefore, when TAI is applied with the re-use of CIDR devices, the protocol should be further determined due to delayed the onset estrus and time of ovulation. **Chiang Mai Veterinary Journal 2013; 11(1): 31-40.**

Key Words: Synthetic progesterone, estrus synchronization, time AI, goat

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Introduction

Estrus 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 & Karatzas, 2004) In small ruminants, intravaginal polyurethane devices (i.e., sponges) impregnated with P4 has already been used, with acceptable success since the 1960s. The CID Rdevice is constructed from natural P4 impregnated medical silicone elastomer molded over a nylon core. (Wheaton, Carlson, Windels, & Johnston, 1993) Currently the CIDR and subcutaneous implants are preferable than sponges because they are easy to use, and CIDR does not absorb or obstruct drainage of vaginal secretions, resulting in less foul-smelling discharge upon removal. (Holtz, 2005; Motlomelo, Greyling, & Schwalbach, 2002; Romano, 2004) However, costs of CIDR are relatively greater than use of sponge and may hinder widespread use of the CIDR. (Souza et al., 2011)

The same CIDR devices, which contain P4, may be used for more than one treatment. (Ungerfeld & Rubianes, 2002) In cattle, CIDR-B (1.9 g of P4) still contained P4 after its use, with the amount dependent on the duration of insertion. (Van Cleeff, Lucy, Wilcox, & Thatcher, 1992) Although not recommended by the manufacturer, re-use device is a common practice in dairy herds. Re-use of P4 intravaginal devices have been reported in cows, (Colazo et al., 2004) ewes (Ungerfeld & Rubianes, 2002) and goats (Oliveira, Guido, & Lima, 2001; Vilariño, Rubianes, & Menchaca, 2011) usually without decreasing fertility rate. Goats that are expressing estrous cycles in typical patterns receiving a new or the re-use CIDR devices showed similar estrus response and pregnancy rates with the second (Oliveira et al., 2001) or third uses. (Vilariño et al., 2011) The most commonly re-use CIDR to synchronize estrus in sheep and goats consist of 5-9 days. (Vilariño et al., 2011) However, there is a paucity of published information regarding the re-use CIDR device in long-term protocol (12-14 day) in sheep and goats. Thus, the re-use of intravaginal devices as a cost-saver, is a possibility to be explored. (Vilariño, Rubianes, Van Lier, & Menchaca, 2010) Therefore, the objective of the present study was to compare the efficiency of first use and re-use synthetic P4 on estrus synchronization and pregnancy rate in goats.

Materials and methods

The experiment was approved by the Animal Ethics Committee of KhonKaen University (Reference No. 0514.1.12.2/67). All goats were vaccinated against foot and mouth disease (FMD), hemorrhagic septicemia (HS) 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 first use and re-use CIDR on estrus synchronization in goats

Animals and estrus synchronization treatments

Twenty-eight nulliparous Thai-native goats, 8-10 months of age with body condition score of 2.5-3.0 and body weight of 17 kg, and were fed a maintenance diet (Committee on Animal Nutrition, National Research Council, 1981) with ad libitum feeding of fresh ruzi grass. Clean water and mineral block were provided throughout the experiment. All female goats were treated with a long-term protocol using an intravaginal CIDR containing 0.3 g of P4 (Eazi-Breed™CIDR®, Pfizer, NY, USA) in place for 14 days. Day 0 was defined as the day of CIDR insertion. Goats were given (IM) 300 IU injection of hCG (Chorulon®, Intervet International B.V., New Zealand) at the day of CIDR removal.Two treatment groups were designed using CIDR devices of first use (new devices, n=14) and re-use CIDR (previously used for 14 d, n=14). The CIDR had been previously used in goats for 14 days in another trial performed on the same farm a month previously. After removal devices were washed with warm water and solution disinfectant, subsequently devices were stored in a dry chamber at room temperature until use.

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 one day before device insertion and ovulation time was determined every 8 h or until 96 h after device removal (if ovulation was not detected). Ovulation was defined as the collapse or disappearance of a large follicle as described by Matemucci and Alessando. (Martemucci & D'Alessandro, 2011)

Estrus detection

Estrus was detected every 8 h for 96 h after the device removal. The onset of the estrus was recorded when the female exhibited the sign of standing to be mounted by the vasectomized buck. (Lertchunha et al., 2012)

Plasma P4 concentrations

Blood samples (5 ml) from day 0 to day 18 (day 0: day of CIDR device insertion) were daily collected via jugular venipunctureinto an EDTA solution. Additional blood samples were acquired on the day of CIDR device insertions (day 0, day 0 + 12 h) and after CIDR removal (day 14, day 14 + 12 h, day 15, day 15 + 8 h, day 15 + 16 h, day 16, day 16 + 8 h, day 16 + 16 h). Samples were immediately placed in ice, transported to the laboratory, and centrifuged at 1500 × g for 15 min. Plasma samples were harvested and stored at -20 °C until assayed. P4 concentrations were determined in duplicate by Enzyme-linked Immunosorbant Assay (ELISA). (Cushwa, Bradford, Stabenfeldt, Berger, & Dally, 1992)

The mean intra- and inter-assay coefficients of variation were 6.5% and 8.6%, respectively.

Experiment 2 Comparison of natural breeding and TAI on pregnancy rate in goats

Fifty-two nulliparous Thai-native goats 8-10 months of age with body condition score of 2.5-3.0 and body weight of 17.5 kgwere estrus synchronized using the first use of 14 days CIDR, and were randomly assigned into 2 treatments. Treatment 1, goats (n=16) were naturally bred with the mature male breeder at 12 h after the onset estrus. Treatment 2, goats (n=36) were cervically TAI at 56 h after CIDR withdrawal with frozen semen from a single proven sire (200×10^6 spermatozoa per dose). Pregnancy rate was determined 70 d after insemination by transrectalultrasonography using a 7.5 MHz transducer (HS-2000, HONDA ELECTRONICS, Japan) and blood samples were taken on 70 days after breeding to confirm pregnancy rate.

Statistical analysis

Time of ovulation, duration of estrus, onset of estrus and follicular diameter at ovulation were analyzed using general linear model (GLM) procedure of SAS (SAS Inst. Inc., Cary, NC). P4 concentrations were compared by a Student t-test. In addition, P4 concentrations were analyzed with a nested analysis of variance with treatment,animal (treatment), and day included in the model. (Navanukraw et al., 2004) Percentages of goats in estrus, ovulation and pregnancy rate were compared between treatments by the Chi-square test. Data were presented as mean ± SEM, and differences were considered significant when $P<0.05$.

Results

Experiment 1 Estrus, ovulation and P4 concentrations

Percentages of goat exhibiting estrus behavior and ovulation were not significantly different between groups (100.0 and 86%, for the first use and re-use; Table 1). However, goats receiving the CIDR device of the re-use exhibited significantly ($P<0.05$) delayed onset estrus and time of ovulation (70.8±1.6 and 93.3±1.8 h) compared to the first use (38.5± 1.5 and 67.0±1.0 h). The distribution of onset estrus indicated that goats receiving first use CIDR exhibited estrus ranging from 32-48 h after CIDR device removal whereas goats receiving re-use CIDR exhibited estrus ranging from 46-94 h. In addition, duration of estrus in goats received the first use CIDR was last longer ($P<0.05$) than those received the re-use. Follicular size at ovulation was similar for devices of the first and re-use CIDR.

On day 0 (before the CIDR insertion), plasma P4 concentrations were similar between the first (2.9 ± 0.2 ng/ml) and re-use (3.4 ± 0.2 ng/ml) treatments (Fig. 1). The maximum concentration of plasma P4 was reached on day 4 after device insertion (6.5± 0.5 and 5.0±0.3ng/ml, for the first and re-use respectively; $P<0.05$) .Although the concentrations were maintained and

Table 1. Effect of first and re-use controlled internal drug release (CIDR) on ovarian responses in goats.

Item	First use (T1)	Re-use (T2)
n = 28	14	14
Estrus and ovulation	14/14 (100%) [a]	12/14 (86%) [a]
Onset estrus* (h)	38.5 ± 1.5 [b]	70.8 ± 1.6 [a]
Time of ovulation* (h)	67.0 ± 1.0 [b]	93.3 ± 1.8 [a]
Duration of estrus (h)	27.0 ± 1.2 [a]	21.3 ± 1.2 [b]
Follicular diameter (mm) at ovulation	6.6 ±0 .1 [a]	6.2 ± 0.2 [a]

*Interval after CIDR withdrawal.

[a, b]Within a row, means with different superscripts differ (P<0.05), Values are mean ± SEM

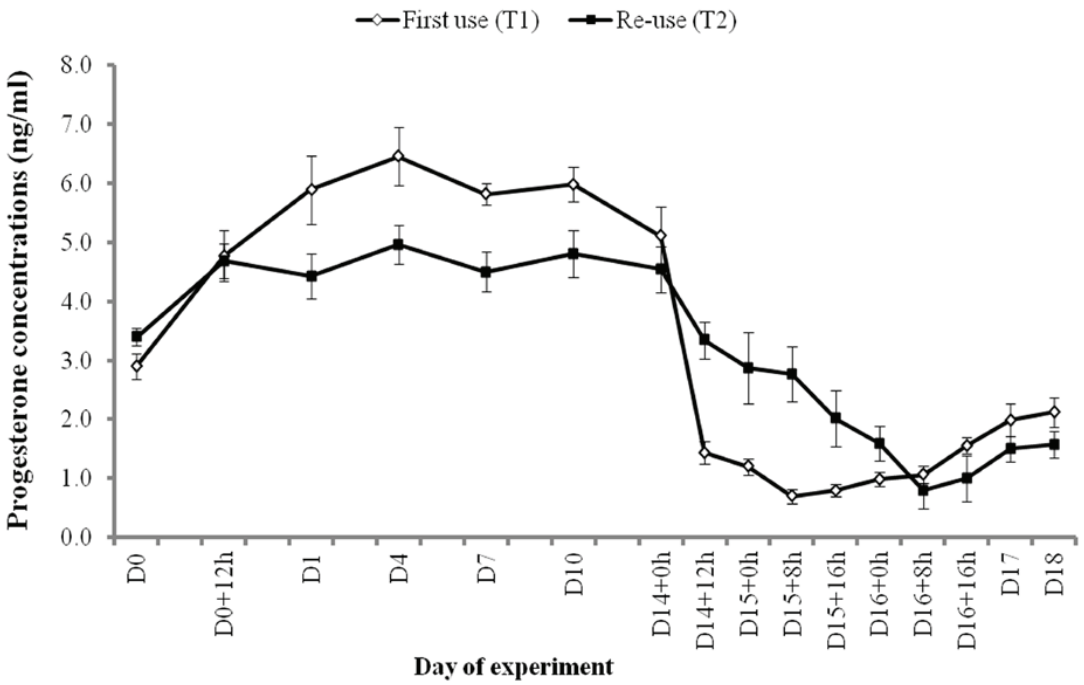

Figure 1. Mean (±SEM) plasma progesterone (P4) concentrations induced by the first and re-use CIDR in goats.

thereafter, the re-use CIDR devices significantly induced lower plasma P4 concentrations than the first use during the time of CIDR insertion. On removal of the CIDR, P4 concentrations decreased rapidly to basal level within 12 h for the first use (1.4 ± 0.2ng/ml) whereas the concentrations gradually decreased to basal level within 40 h (day 15+16 h) for the re-use (1.9 ± 0.2 ng/ml).

Experiment 2 Pregnancy rate

Pregnancy rates confirmed by transrectal ultrasonography was significantly lower for TAI (22.2%) compared with natural breeding (100%) ($P<0.05$). P4 concentrations on day 70 after breeding, remained high (>5 ng/ml) for all pregnant animals from both natural breeding, and TAI group.

Discussion

In our study, percentage of estrus and ovulation did not differ significantly between the first and re-use CIDR treatments. These results are in agreement with many reports of previous studies that have shown thatthe re-use CIDR device was successful in controlling estrus and ovulation in goats. (Souza et al., 2011; Oliveira et al., 2001; Vilariño et al., 2011; Nogueira et al., 2011) Although duration of estrus for the re-use was significantly shorter (21.3±1.2 h) compared to the first use (27.0 ± 1.2 h), it was still in a normal range time. (Lertchunha et al., 2012) The interval from CIDR withdrawal to the onset of estrus and time of ovulation were significantly shorter for the first use compared to the re-use CIDR. Whereas, studies of (Vilariño et al., 2011) and (Nogueira et al., 2011) found the onset estrus and time of ovulation was similar for devices of the first and re-use. These may be attributed to the variation of remaining P4 after its use in a long-term protocol (11-14 day of treatment) (Vilariõ et al., 2011)and it would not suffice in maintaining the levels of P4 required for precise synchronization regimens. Diameters of the ovulatory follicle were similar for the first and re-use devices (6.6±0.1 and 6.2±0.1 mm). These are in agreement with a previous report, two or more follicles in each wave reach >5 mm diameter in goats. (Baril & Vallet, 1990) Plasma P4 concentrations were significantly greater for the new devices compared with devices of re-use. (Souza et al., 2011; Vilariño et al., 2011) On day 0 plasma P4 concentrations were not significantly different between groups. This shows that goats appeared normal physiology of luteal phase, the high P4 concentrations at insert CIDR is a clear indication for cyclicity, because it was assumed ovulations (functional corpus luteum) are present when P4 concentrations are >1.0 ng/ml. (Souza et al., 2011; Fleisch et al., 2012) However, the first and re-use devices ensured that plasma P4 concentrations were maintained higher than 1.0 ng/ml until devices were removal. P4 concentrations induced by both treatments were effective in blocking estrus behavior and ovulation until the devices were removed. (Vilariño et al., 2011) The maximum concentration of plasma P4 was reached on day 4 after device insertion for all groups after CIDR device insertion. However, studies of (Souza et al., 2011) and (Vilariño et al., 2011) found the P4 concentrations were greatest at 6 h and 24 h after device insertion. After devices removal,

P4 concentrations were less than 1.0 ng/ml inthe first use (day 15+8 h) and re-use (day 16+8h). This study differed from previously reported by (Souza et al., 2011) after CIDR device removal P4 concentrations were less than 1.0 ng/ml in all goats of all treatments, this value is desirable because it will allow the occurrence of estrus. Pregnancy rate after natural breeding determined by transrectal ultrasonography was greater when compared with TAI using frozen semen, It was with the high P4 concentrations > 5 ng/ml all pregnant animals.

Conclusion

The present study contributes to new information regarding the re-use of CIDR device for 14 days in goats. At the time of CIDR withdrawal, the re-use CIDR effectively synchronized the estrus and ovulation in goats. Thus, the re-use of CID Rdevice could be used in goat to synchronize the estrus and is therefore a new alternative to reduce cost of the synchrony. TAI could be used in goat, pregnancy rate was lower campared to the natural breeding. However, due to the delayed onset estrus and time of ovulation in re-use group, further reseach focusing on fixed-timed AI using the re-use CIDR is needed for a precise synchronization protocol to improve conception rate.

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