Effects of Two CIDR-based Oestrus Synchronization Protocols on Oestrus Response in Boer Goats

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Abstract

Sixty fertile and healthy female Boer goats were divided equally and randomly into two groups (n=30). The first group received CIDR treatment for 14 days (T14) with 400 IU PMSG and 0.05mg cloprostenol injection (i.m.) prior to CIDR removal and the second group received CIDR treatment for 9 days (T9) with 0.05 mg cloprostenol injection (i.m.) 24 hours before CIDR removal. The number of does with oestrus and the time of oestrus sign were recorded every 6-hour interval and the observation was conducted from 24 hours after CIDR removal and terminated 66 hours after CIDR removal. Blood samples were taken from all of the does before CIDR insertion and 48 hours after CIDR removal. The results showed all 30 does (100%) in T14 and 28 does (93.3%) in T9 came to oestrus. The mean time of does showing signs of oestrus for both treatments were significantly different in tail flagging and standing to be mounted ($P<0.05$). However, the progesterone concentrations between T14 and T9 after 48 h CIDR removal were not significantly different ($P>0.05$). The current study suggests that CIDR treatment for 14 days with 400 IU PMSG and 0.05 mg cloprostenol prior to CIDR removal gave better result in oestrus synchronisation compared to CIDR treatment for 9 days with 0.05 mg cloprostenol given 24 hours before CIDR removal.

Keywords: Oestrus synchronisation, CIDR, oestrus signs, progesterone, goats

Introduction

Oestrus synchronisation is a management practice that manipulates the luteal or follicular phase of the oestrous cycle (Wildeus, 2000; Patterson *et al.*, 2003), thus controlling oestrus and ovulation in cycling females to enable breeding to be conducted within a short period of time (Deutscher, 2010). This practice is applied to farm animals such as cattle (Mapletoft *et al.*, 2003; Patterson *et al.*, 2003; Deutscher, 2010), deer, sheep and goats (Freitas *et al.*, 1996; Wildeus, 2000). The Controlled Internal Drug Release (CIDR) is an alternative device to progestogen sponges for oestrus synchronisation in ruminants. The usage of CIDR provides advantages compared with the sponges such as elimination of foul-smelling mucus discharged upon removal of sponges, lower loss rates, higher percentage of animals coming into oestrus, earlier exhibited oestrus and more compact oestrus (Lynch, 1985; Rhodes and Nathanielsz, 1988). The effectiveness of CIDR in oestrus synchronisation can be increased by co-

treatment with hormones (Oliviera *et al.*, 2001).

Oestrus synchronisation protocols using CIDR vary from insertion of the CIDR for five to 16 days with hormone co-treatment using 100 to 500 IU of equine chorionic gonadotrophin (eCG) or pregnant mare serum gonadotrophin (PMSG) and/or 0.05mg of prostaglandin F2α (PG) (Menchaca and Rubianes, 2001; Whitley and Jackson, 2004; Hashemi *et al.*, 2006). There is no published information about CIDR protocol for 14 days in Malaysia. A shorter duration of CIDR treatment (nine days) with PMSG co-treatment could probably shorten the kidding interval. Thus, the aim of the present study was to compare two oestrus synchronisation protocols using CIDR on oestrus responses in Boer goats in Malaysia.

Materials and Methods

Animals and Oestrus Synchronisation Protocols

The experiment was conducted at Infoternak Farm, Department of Veterinary Services, Sg Siput, Perak. Sixty fertile and healthy female Boer goats were used for the study. The age of the does ranged from one to two years, and all the does had kidded at least once. The does were reared under an intensive management system, fed 300-400g concentrate per head daily and given grass *ad libitum.* The concentrate-grass feed were supplemented with urea mineral salt blocks. The does were provided water *ad libitum.*

Two oestrus synchronisation protocols were evaluated: (1) T14 in which the does were inserted with CIDR (0.3 g of progesterone) for 14 days and received 400 IU of PMSG (FOLLIGON®) and 0.05 mg of cloprostenol (i.m.) (Estrumate®; containing 0.250 mg/ml Cloprostenol as 0.263 mg/ml Cloprostenol Sodium) given prior to CIDR withdrawal and (2) T9 in which the does were inserted with CIDR for 9 days and received 0.05 mg of cloprostenol (i.m.) a day (24 hours) before CIDR withdrawal (Day 0 = day of CIDR insertion). T14 treatment was the sychronization protocol practised on the farm and T9 was selected as it was reported to give the best outcome (Oliveira *et al*., 2001).

Does were randomly assigned into two treatment groups, T14 and T9 (n=30 does per group) and placed in group pens. The mean body weight of the does was 37.81±1.48 kg for T14 group and 40±1.30 kg for T9 group. The experimental design of the study is shown in Figure 1.

Blood samples (5 ml) were collected via jugular vein into plain vacutainer tubes prior to CIDR insertion (P4-pre) and 48 h post CIDR withdrawal (P4-post) (Figure 1). Blood samples were left at room temperature for an hour and stored at 4°C overnight. The samples were centrifuged at 3000 rpm for 15 min and the serum was transferred into 2 ml micro-centrifuge tubes and stored at -20 ºC for progesterone (P4) concentration determination.

Oestrus signs were observed in both T14 and T9 groups for 30 min, at 6-h intervals, starting from 24 h and continued until 66 h post CIDR withdrawal. The oestrus signs recorded were tail flagging, mounting, reddened vulva and standing to be mounted.

Serum progesterone concentrations were determined using enzyme-linked immunosorbent assay (ELISA) kit (Progesterone ELISA, IBL, USA). Firstly, frozen serum samples were thawed at room temperature, and 25 µl of the standard and samples were dispensed into a 96-well microtitre plate using new disposable tips. The plate was incubated at room temperature for 5 min and 200 µl enzyme conjugate was then dispensed into each well. The samples in the well were mixed

thoroughly for 10 sec by gently shaking the plate followed by incubation for 1 h at room temperature. Following that, the contents of the wells were briskly shaken out to remove all the droplets. The plate was rinsed three times with diluted wash solution (30 ml of wash solution diluted with 1170 ml of distilled water). Then, the wells were tapped sharply on absorbent paper to remove all residual droplets.

Approximately, 200 µl of substrate solutions were added in each well and the plate was incubated for 15 min at room temperature. The enzymatic reaction was stopped after 15 min by adding 100 µl of stop solution into each well. Within 10 min, the readings of the serum mixtures were obtained based on the optical density at 400 ± 10 nm using a microtiter plate reader.

Data Analysis

Data on oestrus signs were analysed using Chi-square analysis. The mean time to onset of oestrus sign from withdrawal of CIDR and differences of serum P4 concentrations between the 2 treatment groups were analysed using General Linear Model (SAS 9.0 EDU Edition).

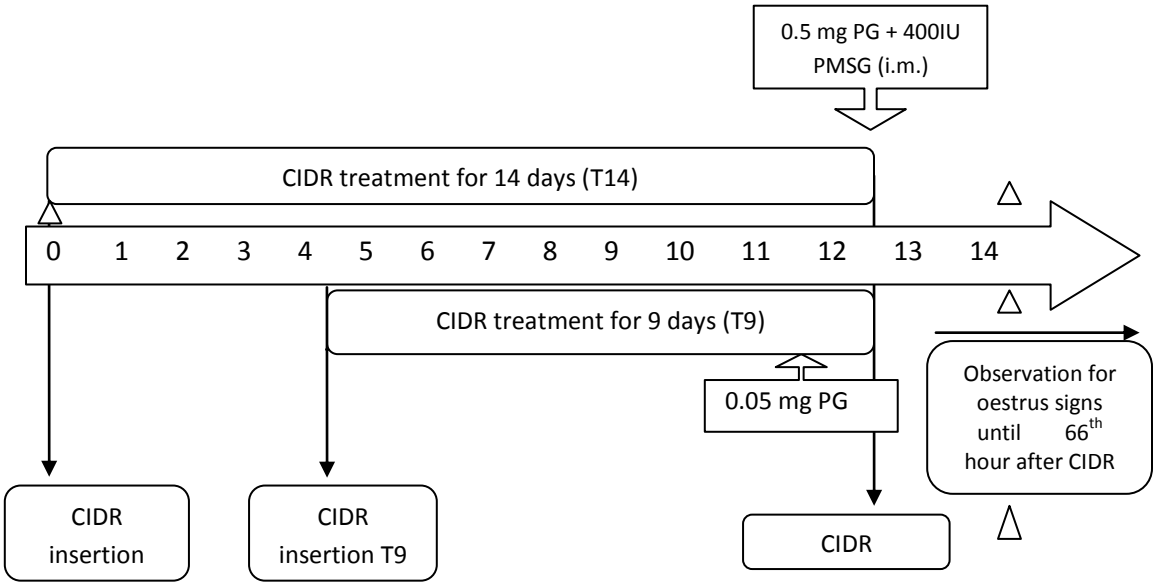

Figure 1: The oestrus synchronisation protocols and the points of blood sample collection

Results and Discussion

All does in T14 group showed oestrus signs within 66 h post CIDR withdrawal. However, in T9 group, two (6.7%) does did not show any oestrus sign or behaviour during the observation period.

Table 1 shows the mean interval from CIDR withdrawal to display oestrus signs in the T14 and T9 treatment groups. The mean interval from CIDR withdrawal to oestrus was significantly shorter ($P< 0.05$) in group T14 compared with group T9. Does in the T14 group stood to be mounted 16.3 h

earlier than the T9 does. Tail flagging in the T14 group was also 8.3 h earlier compared with the T9 group. However, the mean interval from CIDR withdrawal to mounting and reddening of the vulva was not significantly different (P>0.05) between the two groups.

Table 1: The interval from CIDR withdrawal to oestrus sign in Boer does synchronised with T14 and T9 protocols

Interval from CIDR withdrawal to oestrus sign	Synchronisation protocol	
	T14[1] (n=30)	T9[1] (n=28)
Tail flagging (h)	29.6 ± 1.1[a] (n=30)[2]	37.9 ± 2.03[b] (n=28)
Mounting (h)	39.6 ± 2.10[a] (n=15)	47.0 ± 2.86[a] (n=6)
Standing to be mounted (h)	40.7 ± 1.91[a] (n=18)	57.0 ±1.73[b] (n=4)
Reddened vulva (h)	53.7 ± 2.32[a] (n=19)	58.4 ± 2.30[a] (n=15)

[1]T14: CIDR 14 days + PG+ PMSG; T9: CIDR 9 days + PG.
[2]Number of animals for each oestrus sign in parenthesis
[a,b]Means within rows with different superscripts are significantly different (p<0.05)

Table 2 shows the P4 concentrations in serum prior to CIDR insertion (P4-pre) and 48 h post CIDR withdrawal (P4-post). There was no significant difference (P>0.05) in mean serum P4 concentrations at P4-pre and P4-post between T14 and T9 treatment groups. However, for both treatment groups there was significant difference (P<0.05) in the mean serum P4 concentrations between pre- and post-CIDR; mean P4 concentration at P4-post was reduced by 61.8%.

Table 2: Progesterone concentration prior to CIDR insertion (P4-pre) and 48 hours post CIDR withdrawal (P4-post) in Boer does synchronised with T14 and T9 protocols

Progesterone concentration (ng/mL)	Synchronisation protocols	
	T14	T9
P4-pre (ng/mL)	8.3 ± 0.88[a]	8.0 ± 1.64[a]
P4-post (ng/mL)	2.5 ± 0.53[b]	3.7 ± 1.16[b]

[ab]Mean values within the same column or row with different superscripts are significantly different (p<0.05). T14: CIDR 14 days + PG+ PMSG, T9: CIDR 9 days + PG, P4-pre: before CIDR insertion, P4-post: 48 hours after CIDR withdrawal.

Results from previous studies had shown that oestrus synchronisation with CIDR resulted in more compact oestrus in goats compared with medroxyprogesterone acetate progesterone intravaginal sponge (MAP) without PMSG (Rhodes and Nathanielsz, 1988). Both protocols used in this study resulted in very high percentage oestrus response. The percentage of oestrus response in Boer does oestrus synchronised with CIDR for 14 days (T14 group) were similar with those reported by Nasser (2008), although the duration of CIDR treatment was 17 days in Nasser (2008). The

difference between this study and Nasser (2008) is the length of CIDR treatment and probable existence of matured follicles during early treatment which could be a factor contributing to the difference between the two studies, where CIDR treatment for 14 day without gonadotrophin in this study gave better response (93.3%) compared to Nasser (2008) (53.9%) . The presence of matured follicles during the progestagen treatment might prolong the presence of follicle and reduce the oestradiol production during the regression of the corpus luteum (Taft *et al.,* 1996). Gust *et al.* (1984) reported that increasing oestradiol concentration after regression of corpus luteum will promote LH concentrations to rise and trigger the animals to show oestrus.

Inadequate oestradiol secretion can result in failure to display overt signs of oestrus behaviour (silent oestrus) (Romano and Wheaton, 1998; Cardwell *et al.,* 1998; Mustafa *et al.,* 2007). This may explain why oestrus behaviours were not recorded in two does from the T9 group. In addition, exogenous administration of PMSG could result in higher oestrus response (Greyling *et al.,* 1991), and shorten the time to oestrus compared with does that were not oestrus synchronised with PMSG (Freitas *et al.*, 1996; Ucar *et al.,* 2005).

The proportion of does displaying tail flagging and standing oestrus were significantly different ($p<0.05$) between T14 and T9 groups. The significant difference was as expected and supported other studies where the T14 oestrus synchronisation protocol stimulated oestrus earlier (within 36 hour), more compact and shorter time to onset of oestrus (Al-Sobaiyl, 2006; Dogan *et al.,* 2008; Nasser, 2008).

The means of P4 concentration determined the stage of oestrous cycle in the does. Some studies shows the does at luteal phase and oestrus stage had 3.0 - 7.7 ng/mL and 0.1 – 1.0 ng/mL P4 concentration, respectively (Greyling and Niekerk, 1990; Khanum *et al*., 2008; Rahman *et al.*, 2008; Bukar *et al.*, 2012). The P4 concentration for pre and post treatments of T14 and T9 treatment groups was not significantly different. However, the level of P4 concentration in pre-P4 was higher than post-P4 for both treatments which indicated that the does might be in different stages of oestrous cycle before the insertion of CIDR and maybe most of them at the luteal phase. The P4 level in normal oestrous cycle in Dwarf goats during luteal phase peaked at 7.7 ng/mL (Khanum *et al.*, 2008). However, the P4 level in this study was slightly higher compared to Khanum *et al.* (2008) and it might be due to breed differences.

The lower means of P4 level at P4-Post between T14 and T9 compared to P4 level at P4-Pre might be due to the effect of CIDR withdrawal. When the CIDR was in place, the P4 concentration remained high due to exogenous P4 from the CIDR, but at CIDR withdrawal time, the P4 concentration rapidly declined which mimiced the normal condition and subsequently with high oestradiol and low P4 led to the animal to exhibit oestrus behaviour (Khanum *et al.,* 2008; Al-Sobaiyl, 2010).

In conclusion, does with CIDR treatment for 14 days with 400 IU PMSG and 0.05 mg cloprostenol given at CIDR withdrawal tend to shorten the time of oestrus responses and result in more compact onset of oestrus.

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