Progesterone and Luteinising Hormone Profile of Saanen Crossbred Goats Synchronised with CIDR and PMSG

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Abstract

A better understanding of the hormonal profile of oestrus synchronised Saanen crossbreds would guide appropriate timing of Fixed Time Artificial Insemination (FTAI). This study was conducted to determine the progesterone hormone (P4) and luteinising hormone (LH) concentrations from the end of oestrus synchronisation treatment until the estimated time of ovulation. The LH peak would help in estimating the time of ovulation and appropriate time for FTAI. Twenty three Saanen crossbred does aged 2 to 4 y were allocated to three treatment groups: i) CIDR for 14 d and PMSG and PG at CIDR removal (CIDR14++; n=8); ii) CIDR for 9 d and PMSG and PG at CIDR removal (CIDR9++; n=8); iii) CIDR for 9 d and only PG at CIDR removal (CIDR9+; n=7) intramuscularly. Oestrus signs were recorded and blood samples were collected every 4 h beginning at 24 h post CIDR removal for 24 h. The serum samples were analyzed for P4 and LH concentration using ELISA kits. The CIDR9++ does had shorter (p<0.05) interval between CIDR removal and onset of oestrus than the other two treatment groups. All protocols showed declining P4 hormone concentration after 24 h of CIDR removal. The CIDR14++ does had highest mean LH concentration at peak (8.03±2.07 mIU/ml), followed by CIDR9+ does (7.32±1.07 mIU/ml); these values were higher (P<0.05) than the mean for the CIDR9++ does (4.94±0.80 mIU/ml). The LH peak occurred at 42.0±1.15, 41.0±1.91, and 43.0±1.00 h after CIDR removal for the CIDR14++, CIDR9++, and CIDR9+ treatments, respectively. The use of the intravaginal CIDR for oestrus synchronisation may be shortened in goats to 9 d while there was no significant difference in the time of occurrence of LH peak.

Key words: CIDR, LH peak, oestrus synchronisation, Saanen goats

Introduction

Reproductive biotechnology techniques such as oestrus synchronisation, artificial insemination (AI) and embryo transfer have been used to improve meat goat production in many countries including Malaysia. For AI to be successful following oestrus synchronisation, accurate timing of insemination is important. Oestrus detection methods used to determine time of AI can be inconvenient, as well as time and labor consuming. Thus, fixed timed AI (FTAI) protocol that allows for AI without the need of oestrus detection have been developed for cattle (Geary *et al.,* 2001; Bader *et al.,* 2005; Gupta *et al.,* 2008; Perry and Perry, 2008) and sheep (Jafar *et al.,* 2011).

Nowadays, CIDR (Controlled Internal Drug Release device) is widely used to synchronize oestrus, especially in ruminants. Use of CIDR has also been proven to be safe in lactating animals, and their milk is safe for human consumption (Reyes *et al.,* 2012). The long-term implantation of intravaginal devices in the vagina may cause variability in fertility. Evans (2003) and Menchaca *et al.* (2007) reported that the P4 release from the

device may cause prolonged persistence and maturing of the ovulatory follicles resulting in reduction of fertility. In addition, administration of synthetic prostaglandin F2α helps in luteolysis and synchronizing oestrus (Hafez and Hafez, 2000). Usage of PMSG, about 200 IU, may increase the ovulation rate by stimulating ovarian follicles and, subsequently, optimizing litter size (Ritar *et al.,* 1989). These protocols were shown to have high conception rates in goats (Oliveira *et al.,* 2001; Omontese *et al.,* 2013) and resulted in high success rate.

A clearer understanding of the time to oestrus and ovulation following oestrus synchronisation using CIDR in goats would increase conception rate of FTAI and thus, improve local goat production. Appropriate time of FTAI is determined by the time of ovulation occurring towards the end of oestrus period (Van der Westhuysen *et al.*, 1985) or a few hours after the end of standing heat (Shelton, 1978).

Thus, accurate estimation of time to inseminate the does is vital for the success of AI programs. In order to estimate the suitable time for FTAI in Saanen crossbreds raised under humid tropical conditions, determination of the P4 and LH hormonal profiles from end of oestrus synchronisation treatment until ovulation needs to be established. The objective of the study was to determine the P4 and LH concentrations, subsequently to determine the suitable time for AI, in Saanen crossbred goats synchronised using different protocols.

Materials and Methods

Animals and Treatments

The study was conducted at the Universiti Putra Malaysia livestock research farm in Serdang Selangor. Twenty three primiparous and non-lactating multiparous Saanen crossbred goats ranging in age from 2 to 5 years, weighing 35 to 50 kg with body condition score of 3.0 to 4.0, were used in this study. Does were fed with chopped guinea grass (40%) and concentrate (60%) at about 3 % of body weight on the dry matter basis. Water was provided *ad libitum* throughout the study period. The does were randomly divided into three treatment groups, and CIDR-G (EAZI-BREED®, Pfizer, New Zealand Ltd.) containing 0.3 g progesterone either was inserted into the vagina for:

i) 14 d and given 200 IU of PMSG (FOLLIGON®, Intervet, Australia) intramuscularly (CIDR14++; n=8),
ii) 9 d and given 200 IU of PMSG (CIDR9++; n=8),
iii) 9 d without PMSG (CIDR9+; n=7).

All does were also given 0.5 ml of Cloropstenol (PG: Estrumate®, Australia) intramuscularly at CIDR withdrawal.

Oestrus Signs Observation

Oestrus signs were observed for at least 30 min at 4-h intervals from 24 h after CIDR removal and continued for 24 h. Observation was carried out by the same person to minimize bias. Oestrus signs traits recorded were mounting, standing to be mounted, sniffing, vocalization, swollen vulva, vulva discharge and tail wagging.

Blood Sampling

Blood samples were collected from the jugular vein using 21G needle attached to 4 ml plain vacutainer tube (BD® vacutainer, Plymouth, UK). Samples were collected before CIDR insertion, before CIDR removal and 24 h after CIDR removal for progesterone analysis. Blood sampling was also performed at 24 h after CIDR removal and thereafter at 4-h interval for 24 h. The samples were allowed to clot at room temperature and chilled overnight (4 °C)

prior to centrifugation at 500 x g (EBA 8S, Hettich) for 15 min. The serum was transferred into a microcentrifuge tube and stored at -20 °C until further analysis.

Progesterone and Luteinising Hormone Determination

The serum samples collected before CIDR insertion, at CIDR withdrawal and 24 h after CIDR withdrawal were used to determine the P4 concentration. The serial serum samples collected at 24 h after CIDR removal and thereafter at 4-h intervals were used to determine LH concentration. The P4 and LH concentrations were determined using Enzyme-linked Immunosorbent Assay (ELISA) kit (Cusabio®, China). The optical density of each well was read at 450 nm using a microtiter plate reader (Bio-Rad® iMark Microplate reader). The coefficients of variation of intra-assay and inter-assay were 4.4 and 11.7%, respectively, and the minimum detectable levels were 0.2 ng/ml for P4 and 0.24 mIU/ml for LH.

Statistical Analysis

Data of oestrus signs were analyzed using non-parametric Chi-square (χ^2) test of independence. Treatment groups differences on P4 and LH concentrations were analyzed using the General Linear Model (GLM) procedure of the SAS 9.3 software. The level of significance was set at $P<0.05$.

Results and Discussion

There significant differences ($P<0.05$) in the three synchronisation protocols on oestrus signs (Table 1). One doe each from CIDR9++ and CIDR9+ treatment groups were dropped from the analysis due to the loss of CIDR.

The mean time interval between CIDR removal and onset of oestrus was significantly different ($P<0.05$) for the three protocols (Table 2). All does subjected to the CIDR9++ protocol had earlier onset of oestrus and exhibited oestrus signs earlier, followed by treatment groups CIDR14++ and CIDR9+ which was last to display the onset of oestrus.

The serum P4 concentration before CIDR insertion, at CIDR removal and 24 h after CIDR removal are shown in Table 3. For all protocols, the P4 concentration increased at CIDR removal, but decreased by more than 50% about 24 h later. Although the concentration of P4 before CIDR insertion was not significantly different ($P>0.05$) among the three treatment groups, P4 concentration in the CIDR14++ does was higher ($P<0.05$) than the concentration of the CIDR9++ does at CIDR removal and was also higher than both the other synchronisation protocols at 24 h after CIDR removal.

Table 1. Percentage (number) of does expressing different oestrus signs

	Synchronisation protocols		
Oestrus sign	CIDR14++	CIDR9++	CIDR9+
n	8	7	6
Tail wagging	87.5[a] (7)	57.1[b] (4)	0.0[c] (0)
Sniffing	50.0[a] (4)	14.3[b] (1)	0.0[c] (0)
Mounting	37.5[a] (3)	0.0[c] (0)	16.7[b] (1)
Standing to be mounted	0.0[b] (0)	0.0[b] (0)	16.7[a] (1)
Vocalization	37.5[a] (3)	42.9[a] (3)	0.0[b](0)
Swollen vulva	25.0[a] (2)	0.0[b] (0)	0.0[b](0)

[abc]Means with different superscripts within the same row are significantly different (P<0.05)

Table 2. Percentage of does exhibiting oestrus signs and interval from CIDR removal to oestrus

Parameter	Synchronisation protocol		
	CIDR14++	CIDR9++	CIDR9+
Number	8	7	6
No. exhibiting oestrus signs	8	4	2
% exhibiting oestrus signs	100	57.14	33.33
CIDR removal – oestrus onset (h)	34.5 ± 2.5[b]	29.0 ± 1.0[a]	44.0 ± 0.0[c]

[abc]Means with different superscripts are significantly different at P<0.05

Table 3. Mean (± SE) for serum progesterone (P4) concentration (ng/ml) of does synchronised with three protocols

Parameter	Synchronisation protocol		
	CIDR14++	CIDR9++	CIDR9+
Number	5	5	4
Before CIDR insertion	2.96 ± 0.92[a]	2.31 ± 1.20[a]	2.32 ± 0.74[a]
At CIDR removal	10.49 ± 3.22[a]	5.47 ± 1.57[b]	9.35 ± 4.44[a]
Post 12_h CIDR removal	5.38 ± 1.67[a]	1.80 ± 0.04[b]	2.59 ± 0.79[b]

[ab]Means within the same row with different superscripts are significantly different (P<0.05)

Analysis of LH concentrations showed average LH peak occurred at 40 to 44 h after the CIDR removal (Table 4). For the CIDR14++ does, the LH peak occurred at 44 h after CIDR removal and the interval between LH peak and first sign of oestrus was three h earlier than for group CIDR9++.

The time interval from CIDR removal to LH peak was not significantly different (P>0.05) among the treatment groups. Time interval between first sign of oestrus and LH peak was also not significantly different (P>0.05) between the CIDR14++ and CIDR9++ groups; treatment group CIDR9+ could not be compared due to the limited number of does that displayed oestrus. The LH peak concentration was lower (P<0.05) in group CIDR9++ compared to the other two treatment groups.

Table 4. Mean (± SE) duration from CIDR removal and onset of oestrus to LH peak and LH peak concentration for the three synchronisation protocols

Parameter	Synchronisation protocol		
	CIDR14++	CIDR9++	CIDR9+
Number	4	4	4
CIDR removal to LH peak (h)	42.0±1.15[a]	41.0±1.91[a]	43.0±1.00[a]
Onset of oestrus to LH peak (h)	9.33±1.15[a]	12.00±2.83[a]	na
LH peak concentration (mIU/ml)	8.03±2.07[a]	4.94±0.80[b]	7.32±1.07[a]

[ab]Means within a row with different superscripts are significantly different (P<0.05)
na-Not analyzed

Oestrus signs were exhibited in does that were synchronised with CIDR for 14 or 9 d and given PMSG. However, does that were not treated with PMSG failed to show oestrus. Previous studies had shown that administration of PMSG stimulated follicle growth, and resulted in higher oestrus response (Greyling and Van Niekerk, 1990a; Omontese *et al.*, 2013). Similarly, the interval between CIDR removal and onset of oestrus for the CIDR14++ and CIDR 9++ groups which received PMSG were shorter than the CIDR9+ treatment group. This is in agreement with Regueiro *et al.* (1999), who showed that administration of PMSG shortened the interval to oestrus onset. Furthermore, it had been reported that subcutaneous or intramuscular PMSG administration reduced variation in the time to oestrus and time of ovulation among progesterone or progestagen synchronised does (Armstrong *et al.*, 1982; Greyling and van Niekerk, 1990a). This compact of oestrus was not observed in the present study probably due to the small sample size of genetically varied Saanen crossbred does.

Assay of P4 concentration indicated that the mean P4 hormone concentration declined with approaching oestrus. The CIDR14++ does had higher mean P4 concentration at CIDR removal and 24 h after the CIDR removal compared with the other treatment groups. This may be due to the CIDR remaining longer in the vagina and, therefore, it may have caused the P4 hormone concentration in peripheral blood flow to be high even after 24 h of CIDR removal. The hepatic clearance rate of P4 probably increased due to the increased P4 supplementation from the vagina wall tissue (Burke *et al.*, 1996; Parr *et al.*, 1993). A decline in plasma P4 hormone concentration and an elevation of plasma LH concentration stimulated preovulatory follicles to mature

(Gonzalez-Bulnes *et al.*, 2004). The high mean P4 concentration at 24 h post CIDR removal might be lower 32 h post CIDR removal as the average showed lower concentration during onset of oestrus was 34.3±1.9 h in the study on Boer goats by Greyling and van der Nest (2000) and Motlomelo *et al.* (2002). The P4 hormone concentrations ranged between 0.38 to 0.04 ng/ml and 0.3±0.1 ng/ml at 32 h at the onset of oestrus, respectively, in both studies.

The interval between CIDR removal to LH peak was longer than the 37.0±3.6 h interval from FGA sponge removal to LH peak in nulliparous Serrana goats reported by Valentim *et al.* (2006). This difference may be attributed to the different pessary, hormone administration and breed used. The time interval between onset of oestrus to LH peak for CIDR14++ and CIDR9++ were longer than the range of 24 to 32 h found by Khadiga *et al.* (2005) in Damascus goats. On the other hand, Simões *et al.* (2008) reported the time intervals from onset of oestrus to LH peak in monovulatory and polyovulatory goats were shorter (12.2 ± 1.2 and 16.8 ± 1.2 h, respectively); these values approximated the findings in this study. The shorter time interval from onset of oestrus to LH peak may be due to the lower production of estradiol hormone in the blood compared with the study by Sirois and Fortune (1988). This may be due to the differences in the ovulation rate, estrogen secretion rate of follicles and response of animals to PMSG (Greyling and van Niekerk, 1990a). Greyling and van Niekerk (1990b) and Freitas *et al.* (1997) reported similar time interval from the onset of oestrus to LH peak, 10.0±3.5 h and 12.0±4.4 h, respectively. The similarity is probably because of the administration of the PMSG as co-treatment. The ovulation time could be more accurately estimated from onset of oestrus to LH peak compared to the commonly used interval from pessary removal to LH peak because the latter shows higher variation due to the different progestagen treatments. The LH peak determination is important in order to estimate the time of ovulation occurrence. Several studies indicated the time interval from LH peak to ovulation to range from 17.1 ± 1.2 to 20.5 ± 2.4 h (Pierson *et al.*, 2001; Pierson *et al.*, 2003; Simões *et al.*, 2008).

Thus, it could be estimated from the current study that the approximate time of ovulation would probably fall between 55th and 66th h after CIDR removal. When the variation in ovulation time is reduced, the appropriate time for FTAI may be estimated accurately.

Conclusion

The duration of CIDR treatment could be reduced from 14 to 9 d with little effect on the P4 concentration and time of LH peak occurrence. The results from this study also suggest that the suitable time to conduct artificial insemination in goats is between 55 to 66 h after CIDR removal. However, further studies are required to evaluate and compare conception rates among Saanen crossbred goats inseminated using this protocol.

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