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Evaluation of Protocols Based on Synthetic Progesterone and Gonadotropin on Estrus and Ovulatory Response in Thai-Native Goats

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Abstract: The efficiency of protocols based on synthetic progesterone (Medoxy Progesterone Acetate; MAP) and gonadotropins (PMSG/FSH) for estrus synchronization was evaluated in Thai-native goats. Nulliparous goats (n = 36) were randomly assigned to 3 treatments. Treatment 1 (14 days MAP and PMSG); goats were synchronized using 60 mg MAP for 14 days combined with 300 IU PMSG administration 24 h before MAP removal. Treatment 2 (7 days MAP and PMSG); goats received MAP for 7 days combined with 300 IU PMSG administration 24 h before MAP removal and 5 mg $PGF_{2\alpha}$ administration at MAP removal. Treatment 3 (14 days MAP and FSH), goats received MAP for 14 days combined with 3 days of FSH injections in decreasing doses (24 mg total) beginning on day 12 of MAP treatment. Percentage of estrus was 100% detected in goats receiving 14 days MAP and PMSG or 7 days MAP and PMSG whereas 83.3% was observed in goats receiving 14 days MAP and FSH ($p>0.05$). Time from MAP removal to onset of estrus was significantly advanced ($p<0.05$) in goats receiving 14 days MAP and FSH (27.8±2.3 h) when compared to goats receiving 7 days MAP and PMSG (42.0±3.5 h). Duration of estrus was longer in goats receiving 14 days MAP and PMSG (34.0±3.3 h) than those receiving 7 days MAP and PMSG (24.0±2.1 h; $p<0.05$). Results indicate that estrus and ovulatory response of Thai-native goats are not different among protocols, however the use of 14 days MAP and FSH led to shorter interval to onset of estrus than the use of 7 or 14 days MAP and PMSG.

Key words: Estrus synchronization, medroxyprogesterone, gonadotropin, Thai-native goat, ovulatory response, Thailand

INTRODUCTION

Most goats raised in Thailand are native breed due to their environmental and nutritional adaptability. Thai-native goats, classified as a non-seasonal polyestrous breed, exhibit continuous estrous cyclicity throughout the year with first estrus exhibited at 277 days of age with an average estrous cycle length of 20.6 days, estrus lasting 22.5 h and at least one kidding per year. Female Thai-native goats reach puberty when body weight is >17.8 kg (Lertchunhakiat *et al*., 2008). Boonchoo (1997) revealed that the ovulation rate varied throughout the year with the highest rate occurring in October (1.9) and the lowest rate in May and July (1.3) of the year.

Due to the increased popularity of meat goat production in Thailand, estrus synchronization has been widely used and become more important. Synthetic progesterone, i.e., Medroxy Acetate Progesterone (MAP) has been widely used with or without accompanying treatments such as gonadotropins (PMSG, eCG and FSH) or prostaglandin analogs (Menegatos *et al*., 1995; Whitley and Jackson, 2004). MAP is a low-cost synthetic progesterone which has proven as effective hormone for estrus synchronization. Additionally, gonadotropins such as Pregnant Mare's Serum Gonadotropin (PMSG), human Chorionic Gonadotropin (hCG) and Follicle-Stimulating Hormone (FSH) have been also used as a means of inducing ovulation and superovulation in farm animals (Stenbak *et al*., 2003). Fonseca *et al*. (2005) reported that the frequencies of estrus, ovulation and pregnancy were greater in ewes with a combined treatment of progesterone and PMSG than in ewes treated with only progesterone. However, long periods of progestin

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treatment are associated with lower fertility (Vinoles *et al*., 2001; Diskin *et al*., 2002). Thus, decreased periods of progesterone treatment to facilitate management and possibly minimize vaginal discharge and infection and increase fertility have been advocated.

The objective of the present study was to determine the protocol based on synthetic progesterone (MAP) and gonadotropins (PMSG/FSH) on estrus and ovulatory response in Thai-native goats.

MATERIALS AND METHODS

Animals and welfare: This experiment was 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. All experimental procedures were approved by the animal ethic committee of Khon Kaen University. Thirty six nulliparous Thai-native goats, 10-12 months of age with a body weight between 20 and 25 kg were allocated randomly to three treatment groups using a completely randomized design. Animals were fed a maintenance diet with *ad libitum* feeding of fresh ruzi grass. Clean water and mineral block were provided *ad libitum*. Animals 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.

Estrus synchronization and superovulation: Goats were induced into synchronized estrus and ovulation using one of three treatments. Treatment 1 (14 days MAP and PMSG): Animals were inserted with intravaginal sponges containing 60 mg of MAP (Synchrogest esponjas®, Spain) for 14 days and intramusculary injected with 300 IU PMSG (Synchrogest PMSG®, Spain) on day 13 (1 day prior to sponge removal). Treatment 2 (7 days MAP and PMSG + PG): Animals were inserted with intravaginal sponges for 7 days and intramusculary injected with 300 IU PMSG on day 6 (1 day prior to sponge withdrawal) and 5 mg $PGF_{2\alpha}$ (Lutalyse®; Intervet Ltd., Germany) at sponge removal. Treatment 3 (14 days MAP and FSH): Animals were inserted with intravaginal sponges for 14 days and intramusculary injected with FSH twice daily (5, 4 and 3 mg per injection on day 12, 13 and 14, respectively).

Laparoscopic surgery and estrus detection: Goats were injected with 0.075 mg xylazine (Rompun®; L.B.S. Laboratory, Thailand) and 100 mg ketamine hydrochloride (Ketaset®; Wyeth Animal Health, Canada) and laparoscopic surgery was performed to determine numbers of ovarian structures follicles, Corpora Hemorrhagica (CH) and Corpus Luteum (CL) at 24, 48, 72 and 96 h after MAP removal as earlier described for ewes (Luther *et al*., 2007). All visible follicles were then classified by diameter into large (≥7 mm), medium (4-6 mm) or small (≤3 mm) as described (Gonzalez *et al*., 2001). Ovulation rates were then calculated for 24, 48, 72 and 96 h by expressing the number of CH observed at each time point as a percentage of the number of CL at the time of laparoscopies with the assumption that the number of CL represents the total number of follicles ovulated as earlier described by Stenbak *et al*. (2003). Estrus was routinely assessed twice daily (am and pm) by exposing all female goats to vasectomized bucks for 45 min as described by Luther *et al*. (2007).

Blood sampling: Blood samples (7 mL) were collected at times according to treatment protocols via jugular venipuncture into an EDTA solution, then immediately centrifuged at 1500×g for 15 min. Plasma samples were harvested and frozen stored at -20°C until assayed. Progesterone concentrations were determined by Enzyme-linked Immunosorbant Assay (Cushwa *et al*., 1992). The intra-assay coefficient of variation was 5.3% and assay sensitivity was 0.025 ng mL^{-1}.

Statistical analyses: Average time to estrus duration of estrus and numbers of follicles, CH and CL were analyzed using General Linear Model (GLM) procedure of SAS (2001). Ovulation rates were analyzed by the Chi-square test. A p-value 0.05 was taken to denote statistical significance.

RESULTS AND DISCUSSION

Using laparoscopy, no significant difference was observed in total numbers of visible follicles among treatments at each time points ($p>0.05$, Table 1). These results are in agreement with many reports of earlietr studies that have shown that progesterone with exogenous PMSG or FSH can promote ovarian activity and follicular development in does (Greyling and Van Niekerk, 1990; Menegatos *et al*., 1995; Greyling and Van der Nest, 2000). Treatment of 14 days MAP and FSH had greater number of ≥7 mm follicle at 24 and 48 h than other treatments ($p<0.01$) due to the shorter biological half-life of FSH (2 h) compared with PMSG (20 h) (Armstrong *et al*., 1983; Chao *et al*., 2008). Driancourt (2001) described a reduction of size of ovulatory follicles in FSH or PMSG stimulated ewes in contrast to the non-superovulated animals.

Table 1: Number of visible follicles at different time after MAP removal

Items	14 days MAP and PMSG	7 days MAP and PMSG	14 days MAP and FSH	p-value
Follicle <3 mm at 24 h	2.08±0.50	2.75±0.49	1.25±0.43	0.09
Follicle <3 mm at 48 h	3.00±0.52	2.08±0.29	1.92±0.38	0.15
Follicle <3 mm at 72 h	3.50±0.51	2.83±0.34	2.33±0.28	0.12
Follicle <3 mm at 96 h	3.50±0.34	2.92±0.26	2.75±0.48	0.33
Follicle 4-6 mm at 24 h	4.42±0.90	3.58±0.56	3.00±0.83	0.44
Follicle 4-6 mm at 48 h	4.33±0.69	4.75±1.05	3.33±0.75	0.48
Follicle 4-6 mm at 72 h	3.33±0.51	3.92±0.36	4.25±0.65	0.46
Follicle 4-6 mm at 96 h	3.50±0.51	3.75±0.25	4.75±0.76	0.25
Follicle ≥7 mm at 24 h	1.17±0.32^{b}	1.00±0.25^{b}	3.33±0.62^{a}	0.01
Follicle ≥7 mm at 48 h	1.83±0.39^{b}	1.83±0.24^{b}	3.92±0.83^{a}	0.01
Follicle ≥7 mm at 72 h	2.42±0.34	1.92±0.38	3.75±1.35	0.29
Follicle ≥7 mm at 96 h	2.50±0.31	2.00±0.43	2.92±1.47	0.77
Total number of follicle at 24 h	7.67±0.95	7.33±0.71	7.58±1.69	0.98
Total number of follicle at 48 h	9.17±0.61	8.67±1.04	9.17±1.67	0.94
Total number of follicle at 72 h	9.25±0.78	8.67±0.86	10.33±2.08	0.69
Total number of follicle at 96 h	9.50±0.66	8.83±0.76	10.42±2.31	0.74

^{a,b}different superscripts in the same row indicate significant difference among treatment groups

Table 2: Number of Corpora Haemorhagica (CH), Corpora Lutea (CL) and percentage of ovulation after MAP removal

Items	14 days MAP and PMSG	7 days MAP and PMSG	14 days MAP and FSH	p-value
Number of CH				
24 h	0.83±0.11	0.67±0.22	0.67±0.22	0.54
48 h	1.67±0.22	1.42±0.15	1.08±0.31	0.68
72 h	2.92±0.34	2.25±0.25	1.75±0.43	0.28
96 h	3.33±0.33	3.17±0.27	2.50±0.60	0.49
Number of CL at 96 h	3.33±0.33	3.17±0.27	2.50±0.60	0.49
Percentage of ovulation				
24 h	29.72	21.53	28.75	>0.05
48 h	50.28	45.83	43.13	>0.05
72 h	88.19	70.83	73.13	>0.05
96 h	100.00	100.00	100.00	-

^{a,b}Different superscripts in the same row indicate significant difference among treatment groups

Table 3: Effect of treatments on estrous response, time from MAP removal to estrus and duration of estrus

Items	14 days and PMSG	7 days MAP and PMSG	14 days MAP and FSH	p-value
Estrous response (%)	100.0 (12/12)	100.0 (12/12)	83.3 (10/12)	0.12
Time to estrus (h)	36.0±4.2^{ab}	42.0±3.5^{a}	27.8±2.3^{b}	0.03
Duration of estrus (h)	34.0±3.3^{a}	24.0±2.1^{b}	28.0±1.7^{ab}	0.02

^{a,b}Different superscripts in the same row indicate significant difference among treatment groups

The mean number of CH and CL was not different among treatments (Table 2). The ovulatory follicles of goat receiving all treatment groups were completely ovulated within 96 h. At this time period, the CL appeared on ovaries while there were not represented of CH more. Thus, ovulation rates were expressed as criteria of ovulatory response. The majority of the ovulations occurred during 48-72 h after MAP withdrawal. Similarly, Walker *et al.* (1986) demonstrated that 79% of ovulations occurred between 54 and 66 h after progesterone removal and FSH injections in Merino sheep. Frequency of ovulation did not differ among treatments. About half of the ovulations in the current study occurred before 48 h. Percentage of ovulation was completely occurred by 96 h (100%). These results demonstrated that the uniformity of ovulation in hormonal treated goats was similar to sheep treated with Syncro-Mate-B (SMB) for 14 days and FSH (Stenbak *et al.*, 2003). However, approximately 30% of the follicles still remain unovulated in ewes receiving 14 days SMB and FSH. Thus, ovulation in this study was not spread over an expected time period.

Several studies have evaluated time of insemination after MAP, SMB or CIDR combination with PMSG-or FSH-treatments and male exposure. For goats, the optimal time to inseminate was between 48-52 h after intravaginal progesterone removal (Lopez-Sebastian *et al.*, 2007). For Merino and Suffolk ewes, insemination between 48-50 h after progesterone removal resulted in high conception rate. Therefore, the optimum timing of insemination is related to the timing of the injection of PMSG in relation to sponge removal (Whitley and Jackson, 2004).

The percentage of estrus did not differ significantly among groups (Table 3). None of the goats exhibited estrus while the MAP was in place indicating that 60 mg MAP was sufficient to suppress the preovulatory discharge of pituitary gonadotropins (Romano, 2004). Half-dose of intravaginal MAP did not affect the efficiency of estrus synchronization in Boer and Indigenous goats (Greyling and Van der Nest, 2000). Thus, 94.4% of the goats were in estrus following a 7/14 days MAP treatment. These results are in agreement with those of Motlomelo *et al.* (2002) comparing 16 days MAP, Fluorogestone Acetate (FGA) and Controlled Internal Drug Release (CIDR) without gonadotropins in Boer and South African indigenous goats. Romano (2004) reported the estrous response of Nubian goats was 100% when MAP, FGA or CIDR was applied to synchronize estrus during breeding season. Goats receiving 14 days MAP and FSH exhibited estrus earlier ($p<0.05$) when compared to goats receiving 7 days MAP and PMSG but did not differed to goats receiving 14 days MAP and PMSG. Difference could be mostly due to difference in

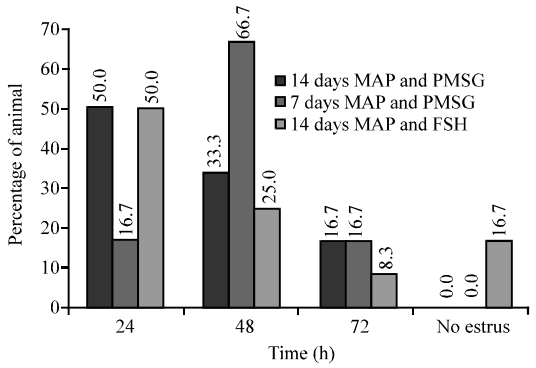

Fig. 1: Estrous response at different times after MAP removal

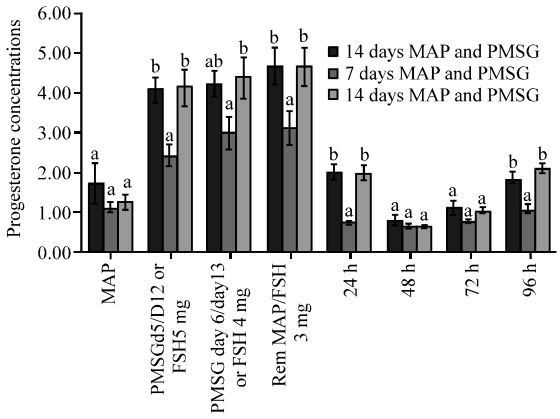

Fig. 2: Plasma progesterone concentrations of goats during the time of MAP and PMSG/FSH treatments. Means with different letters differ ($p>0.05$)

rate of absorption and metabolization of MAP (Romano, 2004). The distribution of estrus indicated that 50% of goats receiving 14 days MAP exhibited estrus within 24 h after MAP removal whereas the majority (66.7%) of goats receiving 7 days MAP exhibited later at 48 h (Fig. 1).

Duration of estrus was longer in goats receiving 14 days MAP and PMSG (34.0±3.3 h) than goats receiving 7 days MAP and PMSG (24.0 ± 2.1 h; $p<0.05$). In the present study, $PGF_{2\alpha}$ was used in goats receiving 7 days MAP and PMSG as a luteolytic agent for the elimination of remnant CL, since exogenous progestin does not affect production of progesterone by the CL (Romano, 1996). Therefore, $PGF_{2\alpha}$ might affect duration of estrus. These results are in agreement with those of Selvaraju *et al.* (1997) and Greyling and Van der Nest (2000) but are in contrast to those of Motlomelo *et al.* (2002), Romano (2004) and Fonseca *et al.* (2005). The mean duration of the induced estrus (29.5±2.4 h) obtained in this current study is comparable to the natural duration of estrus in Boer goats (37.4±4.8 h; Greyling, 1988) and in South African indigenous goats (33.2±3.4 h; Motlomelo *et al.*, 2002).

Estradiol is the factor responsible for the induction of estrous behavior and the LH surge. Although, the number of follicle 1-3 and 4-6 mm was not significant different among treatments, the number of follicle >7 mm at 24 and 48 h in goats receiving 14 days MAP and FSH was greater ($p<0.05$) than other treatments. This observation could be hypothesized that estradiol obtained in follicle >7 mm in goats receiving MAP and FSH probably was greater than goats receiving MAP and PMSG then triggered estrous expression sooner than other treatments (Romano, 1996).

The average progesterone concentrations at the MAP insertion were 1.74, 1.14 and 1.26 ng mL^{-1}, respectively (Fig. 2). Plasma progesterone concentrations increased after MAP insertion and were greater and remained higher ($p>0.05$) before MAP removal in goats receiving 14 days MAP and PMSG/FSH than goats receiving 7 days MAP and PMSG. The progesterone concentrations declined rapidly after MAP removal. The pattern of progesterone concentrations was similar to the report of Romano (1998, 2002) and Motlomelo *et al.* (2002).

CONCLUSION

Treatment with MAP plus PMSG or FSH resulted in similar response of estrus and ovulation. However, time to estrus of goats receiving 7 days MAP and PMSG occurred later than goats receiving 14 days MAP and FSH. Thus, application of these protocols for estrus synchronization and time of AI should be in consideration of each protocol.

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