



RESEARCH ARTICLE

Corpora Lutea Diameter, Plasma Progesterone Concentration and Follicular Development in PGF_{2α} and CIDR Estrus Synchronized Goats

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ABSTRACT

The current study compares the number and diameter of the corpora lutea (CL), plasma progesterone concentrations and follicular development in PGF_{2α} and CIDR synchronized estrus cycle, their subsequent estrus cycles, and in unsynchronized, naturally cycling Boer x Feral crossbred goats. The PGF_{2α} group was synchronized with a double intramuscular injection of 125 µg cloprostenol 11 days apart, the progesterone group was synchronized with CIDR left in place for 17 days, while the third group was not synchronized and served as control. All the estrus synchronized goats ovulated and formed normal CL while 25% in the subsequent estrus cycle and 50% of the naturally cycling goats did not ovulate and hence might be a cause of reduced fertility in the goats. The diameter of the CL, and the plasma progesterone concentration between the PGF_{2α} synchronized (11.9±0.5 mm; 3.51±0.19 ng/ml) and their subsequent estrus cycle (12.0±0.4 mm; 3.22±0.71 ng/ml), as well as between CIDR synchronized (12.3±0.4 mm; 5.98±1.11 ng/ml) and subsequent estrus cycle (12.5±0.8 mm; 4.25±1.37 ng/ml) were not significantly different (P>0.05) but were higher than in the unsynchronized goats (9.3±3.8 mm; 2.99±1.64 ng/ml). The day of emergence and duration of follicular waves, as well as the maximum diameter attained by the largest follicle in the follicular waves was unaffected irrespective of whether PGF_{2α} or CIDR was used for estrus synchronization. This indicated that the morphology and function of the CL did not influence these aspects of follicular development in non-seasonally polyestrous Boer crossbred goats in the humid tropics.

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INTRODUCTION

The use of real-time ultrasonographic techniques to monitor the pattern of antral follicular development in cattle and goats has increased the understanding of the dynamics of follicular development, growth, dominance and atresia as well as the ovulatory process (Simoes *et al.*, 2006; Aerts and Bols, 2010). In goats, the first description on the pattern of antral follicular development during estrus cycle was reported by Ginther and Kot (1994). Later, the patterns of antral follicular development during interovulatory intervals were described in goats synchronized with either intravaginal progestagen sponges (Fernandez-Moro *et al.*, 2008) or two injections

of prostaglandin (PGF_{2α}) (Simoes *et al.*, 2006; Vazquez *et al.*, 2010). These previous studies showed that the methods of estrus synchronization (synch) resulted in morphological and functional changes in the induced CL, thus affected the subsequent follicular development during the interovulatory interval.

The ultrasonographic appearance of a corpus luteum is a reliable parameter that could be used to assess luteal function in goats (Orita *et al.*, 2000; Simoes *et al.*, 2007). Vazquez *et al.* (2010) compared the morphology and progesterone secretion of CL from spontaneous (natural) estrus cycle of Anglo-Nubian goats with CL from PGF_{2α} synchronized estrus cycle. It showed that the CL from PGF_{2α} synchronized goats were larger but secreted less

progesterone than the CL from the natural cycles. Similarly, follicular development during interovulatory interval showed that the estrus synchronized goats had higher number of follicles, larger maximum diameter of the follicles during the first and second follicular waves than the spontaneous cycling goats (Vazquez *et al.*, 2010). Fernandez-Moro *et al.* (2008) also reported that goats that were estrus synchronized with flugestone acetate (FGA) had a higher number of preovulatory follicles compared with PGF_{2α} synchronized goats. Furthermore, Baby and Bartlewski (2011) showed that progesterone from the CL is an important endocrine signal which regulates the periodicity of follicle stimulating hormone (FSH) peaks and the emergence of follicular waves in cyclic ewes. However, it is unclear if the number and diameter of the induced CL, plasma progesterone concentration and the pattern of follicular development are different between the PGF_{2α}, and CIDR synchronized, and their subsequent estrus cycles compared with unsynchronized estrus cycles. Thus, this study was conducted to analyse and compare CL number, diameter, progesterone level, and follicular development during the interovulatory period of PGF_{2α} and CIDR synchronized estrus cycles, their subsequent estrus cycles, and unsynchronized, naturally cycling goats.

MATERIALS AND METHODS

Experimental design: This experiment was conducted on a private goat farm in Kuang, Malaysia (Lat: 3° 15N and Long: 101° 32' 60E) using a total of 24 Boer x Feral goats between 3 and 4 years old, and with average body weight of 35 ± 2.7 kg. Estrus behavior was observed in the goats throughout the year. The goats had a median body condition score (BCS) of 3 out of 5 (BCS 1=very thin, BCS 3=moderate, BCS 5=Obese) as the BCS of these goats were determined based on the amount of muscles and fats around the ribs and loins (Burkholder, 2000). Estrus behavior was observed in all the goats within one month that preceded the study (February 2009). The ambient temperature and relative humidity during the 3 months experimental duration (February to April, 2009) ranged from 20 to 35°C and 67 to 83%, respectively. The goats were fed with a mixed ration comprising soya bean meal and oil palm leaf silage based on 3% their body weight and supplemented with commercial ruminant pellets. The total feed given contained 16% crude protein and 10MJ/kg of metabolizable energy. Water and salt licks were provided *ad libitum*.

The goats were divided equally into three groups: PGF_{2α} synchronized group, the CIDR synchronized group, and an unsynchronized (control) group. In the PGF_{2α} group, estrus was synchronized with two intramuscular injections of 125 µg cloprostenol (Estrumate™, Schering-Plough, Australia) given 11 days apart (Cruz *et al.*, 2005). In the CIDR group, the goats were synchronized with controlled internal drug release (CIDR, EAZI-BREED™, New Zealand), which contained 300 mg progesterone and inserted into the vagina and left in place for 17 days (Montlomo *et al.*, 2002).

Blood sampling and ultrasonography: Transrectal ultrasonographic examination of the ovaries was performed in

all the goats once daily using a real-time B-mode ultrasound scanner (Aloka, 500 SSD, Japan), with a transrectal 7.5-MHz linear array probe (UST-660-7.5 model); until a complete estrus cycle of normal length (19-22 days) was recorded. Blood samples (5 ml) were collected from each goat prior to ultrasound scanning, twice a week at interval of 3-4 days via the jugular vein into heparinized vacutainer tubes. Blood samples were centrifuged at 1006 g for 15 min, plasma was aspirated and stored at -20°C until assay. Plasma progesterone concentration was measured using radioimmunoassay kit (PROG-CTK-4; DiaSorin, Italy). Inter- and intra assay coefficients of variation (CV) were 5.9 and 4.0% respectively, and analytical detectable limit (sensitivity) was 0.05 ng/ml.

For the two synchronized groups, ultrasonographic monitoring of ovarian follicular development commenced 24 hrs after the second PGF_{2α} injection or CIDR withdrawal, while goats in the control group were scanned from the beginning of the study until a complete natural cycle was recorded. The follicles >3 mm diameter were measured using the ultrasound scanner's built-in electronic caliper.

The CL were recorded and monitored as soon as they became distinguishable by day 4 after ovulation according to a previously described method (Chao *et al.*, 2008). The mean maximum CL diameter and progesterone concentration was calculated as the average of 3 successive daily recordings on days 9, 10 and 11 after ovulation at maximum CL diameter and progesterone concentration. Follicular development parameters analyzed included day of follicular wave emergence from ovulation, duration of the follicular wave (days) and maximum diameter attained by the largest follicle in a wave. The follicular wave terminologies used were according to previously described methods; a follicular wave was defined as a cohort of two or more 3 mm diameter antral follicles that emerged within 48 hours and grew to at least 5 mm in diameter before regression or ovulation (Ginther and Kot, 1994; Simoes *et al.*, 2006). Emergence of a wave was the day when 3-mm follicles were first detected, while the duration of a wave was the interval between wave emergence of a cohort of 3 mm diameter follicles and the regression of the follicles to 3 mm in diameter (Simoes *et al.*, 2006).

Data analysis: The number and diameter of the CL were expressed as mean±SE. The mean diameter of the CL and plasma progesterone concentration between the synch and subsequent estrus cycles of the PGF_{2α} and of the CIDR synch groups were compared using paired t-test, while the synch, subsequent estrus cycles and the unsynchronized cycles were compared using analysis of variance (ANOVA). Kruskal-Wallis non-parametric analysis of variance (ANOVA) was used to analyze the mean time of emergence and duration of follicular waves, and maximum size attained by the largest follicles of sequential waves of the synch and unsynchronized groups. The SPSS statistical software version 17 (SPSS Inc.) was used for all data analyses.

RESULTS

The number of goats with one corpus luteum versus two CL and the number of non-cycling goats during the synchronized and subsequent estrus cycles are shown in Table 1. All the goats in the PGF_{2α} and CIDR synchronized groups ovulated and developed a CL during the interovulatory interval. However, two goats each (25%) from the subsequent natural cycle of the PGF_{2α} and CIDR synchronized groups did not ovulate (not cycling), while four goats (50%) in the unsynchronized (control) group were not cycling.

The mean CL diameter and progesterone concentration recorded during the inter-ovulatory intervals are shown in Table 2. The mean CL diameter and progesterone concentration of the synch groups were not different ($P>0.05$) from the mean CL diameter recorded during the subsequent natural estrus cycle but were significantly larger ($P<0.05$) than the mean CL diameter and progesterone concentration in the unsynchronized group.

Table 3 shows that the time of emergence and duration of the follicular waves from ovulation and duration of the follicular waves among the synchronized, subsequent estrus cycle and in the unsynchronized group were not significantly different ($P>0.05$). Similarly, Table 4 shows that the mean maximum diameter attained by the largest follicle in the follicular waves among the synch and in the unsynchronized groups was not significantly different ($P>0.05$).

DISCUSSION

In the current study, CL were formed in all the goats in synch groups, while in each of two synchronized groups two non-cycling goats (25%) were observed in the

subsequent natural cycle and four goats in the unsynchronized groups (50%) were not cycling. This indicated that estrus synch either with PGF_{2α}, or CIDR might contribute to improve regular cyclicity in these goats and that up to 50% of the spontaneously cycling goats studied might have failure of ovulation despite the observation of regular estrus behavior. The finding of the current study implies that abnormal ovarian cyclicity in the Boer goats and their crosses with other exotic goat breeds that have currently been introduced into many tropical countries such as Malaysia might be a significant cause of infertility and low success rates in the breeding programs of sheep and goat breeds (Kosgey *et al.*, 2006). In a previous report, approximately 30% of Boer goats in South Africa had long estrus cycles (40 to 60 days) except during the period of peak sexual activity in April and May (autumn) compared with the other months of the year, although there was no period of complete anestrus (Greyling, 2000). At present, there is no report of a period of reduced sexual activity or the prevalence of abnormal ovarian cyclicity among non-seasonal polyestrous goats in Malaysia.

In the current study, the mean CL diameter and progesterone concentrations of the synch groups were not significantly different ($P>0.05$) with the mean CL diameter recorded during the subsequent estrus cycle, indicating that the CL diameter and progesterone concentration in the synchronized and subsequent estrus cycles were similar. However, the mean CL diameter and progesterone concentrations in the synch groups were significantly higher ($P<0.05$) than the mean CL diameter and the progesterone concentration in the unsynchronized group. Previous studies showed that a significant positive correlation existed between the diameter of CL and progesterone concentration in goats (Orita *et al.*, 2000;

Table 1: Number (%) of CL in synch, subsequent and unsynchronized estrus cycles in goats

Method of estrus synch	Synchronized estrus cycle			Subsequent estrus cycle		
	No CL	One CL	Two CL	No CL	One CL	Two CL
PGF _{2α}	-	4 (50)	4 (50)	2 (25)	2 (25)	4 (50)
CIDR	-	3 (37.5)	5 (62.5)	2 (25)	4 (50)	2 (25)
Unsynchronized* (control)	-	-	-	4 (50)	2 (25)	2 (25)

*The unsynchronized goats were monitored for natural estrus cycle only.

Table 2: Mean±SE (mm) diameter of the corpora lutea and plasma progesterone concentrations (ng/ml) in PGF_{2α}, CIDR synchronized and unsynchronized estrus cycles in goats

Method of estrus synch	Diameter of CL		Progesterone concentration	
	Synchronized	Subsequent estrus cycle	Synchronized	Subsequent estrus cycle
PGF _{2α}	11.9±0.5 ^{ax}	12.0±0.4 ^{ax}	3.51±0.19 ^{ax}	3.22±0.71 ^{ax}
CIDR	12.3±0.4 ^{ax}	12.5±0.8 ^{ax}	5.98±1.11 ^{ax}	4.25±1.37 ^{ax}
Unsynchronized (control)*	9.3±3.8 ^b		2.99±1.64 ^b	

Values with different superscripts within rows (x, y) and between rows within columns (a, b) denote statistical significance ($P<0.05$); *The unsynchronized goats were monitored for natural estrus cycle only.

Table 3: Mean±SE day of emergence and duration of follicular waves in Boer crossbred goats synchronized with PGF_{2α}, CIDR, their subsequent estrus cycles and in unsynchronized group

Wave Number	Follicular wave parameter (days)	Method of estrus synch				P-value	
		PGF _{2α}		CIDR			Unsynchronized Group
		Synchronized estrus cycle	Subsequent estrus cycle	Synchronized estrus cycle	Subsequent estrus cycle		
1	Day of emergence	1.7±0.4	2.5±0.7	1.3±0.3	1.5±0.4	1.5±0.3	P=0.59
	Duration	8.0±0.8	6.9±0.3	7.6±0.6	7.5±1.2	5.8±0.8	P=0.24
2	Day of emergence	9.6±1.0	8.6±0.3	8.5±0.8	8.4±1.3	6.0±1.4	P=0.08
	Duration	5.0±0.2	6.1±0.7	7.1±1.1	5.5±0.8	5.4±0.5	P=0.16
3	Day of emergence	14.3±0.9	14.4±0.7	14.4±0.3	12.0±2.1	10.1±1.5	P=0.07
	Duration	5.7±0.6	5.4±0.4	6.0±0.5	7.3±1.1	6.4±0.5	P=0.53

Table 4: Mean±SE maximum diameter (mm) attained by the largest follicle in the three follicular waves of PGF_{2α} or CIDR synchronized, and unsynchronized estrus cycles

Number of waves	Method of estrus synch			P-value
	PGF _{2α}	CIDR	Unsynchronized	
1	7.3±0.5	8.7±0.9	7.0±0.3	0.27
2	5.9±0.5	6.8±0.3	6.7±0.3	0.24
3	7.0±0.4	7.4±0.5	6.7±0.5	0.24

Chao *et al.*, 2008). Furthermore, Simoes *et al.* (2007) reported that plasma progesterone concentration was higher in goats with two CL (6.4±3.7 ng/ml) compared with goats having only one CL (5.4±3.0 ng/ml). These findings might explain the lower progesterone concentration in the naturally cycling goats with lower mean CL diameter found in the current study compared with the synch groups. Our findings are also in agreement with Letelier *et al.* (2011), who reported that in ewes, secretion of progesterone was significantly higher in cloprostenol synchronized ewes compared with FGA synchronized or naturally cycling ewes. Vazquez *et al.* (2010) also reported that there were more double ovulations and the CL of the goats were larger in the PGF_{2α} synch goats compared with the naturally cycling goats. However, in contrast with our findings, Vazquez *et al.* (2010) reported that the CL from the naturally cycling goats secreted higher progesterone than the PGF_{2α} treated goats due to defective preovulatory follicular development. This resulted in reduced functionality and progesterone secretion from the induced CL of the PGF_{2α} synch goats compared with the naturally cycling goats. Thus, the finding of the current study infers that the reduced functionality did not influence the CL diameter and progesterone concentration in the PGF_{2α} synch goats. Furthermore, the fewer number of CL in the unsynchronized group found in the current study might also have contributed to the lower mean CL diameter and progesterone concentration compared with the synch group.

In the current study, the mean day of emergence of the follicular waves, the duration of the follicular waves and the maximum diameter of the follicles among the synch groups and in the unsynchronized group were not significantly different. Previous studies have shown that in cattle (Adams *et al.*, 1992), ewes (Leyva *et al.*, 1998) and goats (Gonzalez-Bulnes *et al.*, 2005; Cueto *et al.*, 2006), exposure of the developing follicles to low progesterone concentration during the follicular growth increased the maximum diameter attained by the follicles. However, in the current study, the lower mean CL diameter and progesterone concentration in the unsynchronized group did not result in significant differences in the pattern of follicular development. Cueto *et al.* (2006) had reported that the day of follicular wave emergence was not significantly different between and within Anglo-Nubian and Saanen breeds. Similarly, there were non significant differences in day of follicular wave emergence between two natural estrus cycles of Anglo-Nubian goats (Filho *et al.*, 2007).

The implication of the current finding on the pattern of follicular development is that estrus synch with PGF_{2α} or CIDR in goats could be successfully followed up by estrus re-synch immediately afterwards with either hormone and with comparable percentage estrus response, ovulation and pregnancy rates as was recorded in cows (Sani *et al.*, 2011).

Thus, with the increased interest and application of artificial reproductive technology such as AI in goat production, we anticipate that a successful outcome could result from re-synch of estrus in unmated goats or those that returned to estrus after timed artificial insemination (TAI) as is practiced in cows.

In conclusion, estrus synchronization increased the number of cycling goats. The diameter of the CL, plasma progesterone concentration and follicular wave pattern during the interovulatory period of synchronized estrus cycles and their subsequent estrus cycles were not significantly different irrespective of whether PGF_{2α} or CIDR is used. On the other hand, the CL diameter and progesterone concentration was lowest in the unsynchronized goats. However, the day of emergence and duration of follicular waves, as well as the maximum diameter attained by the largest follicle in the follicular waves was unaffected, indicating that the morphology and progesterone secreted from the CL did not influence these aspects of follicular development in non-seasonally polyestrous goats in the humid tropics.

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