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Effect of dosage and duration of altrenogest treatment on follicular development and ovulation in sows*

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Abstract: The aim of this study was to investigate the effect of altrenogest (ALT) treatment on follicular development and ovulation in sows. Sows (parity 6–9; D0: weaning day) were assigned to four treatments as follows: control (no ALT treatment; n = 20), ALT-4–0 (ALT 20 mg/day, 5 days; D-4–D0; n = 20), ALT-4–2 (ALT 20 mg/day, 7 days; D-4–D2; n = 23), and 2ALT-4–2 (ALT 20 mg twice a day [40 mg/day], 7 days; D-4–D2; n = 24). The sows in each group were further divided into three groups based on slaughter periods: day 1 (DF1) and day 4 (DF4) of the follicular phase, and after the initiation of estrus. The number of follicles was reduced on DF4 compared to DF1 ($P < 0.001$). On DF1, follicular size (FS) of the control was larger than that of ALT-4–0, ALT-4–2, and 2ALT-4–2, but on DF4, FS of ALT-4–2 and 2ALT-4–2 was larger than that of the control and ALT-4–0 ($P < 0.001$). The coefficient of variation of FS on DF4 was smaller in ALT-4–2 compared to the control and 2ALT-4–2 ($P < 0.05$). Ovulation rate was similar between groups ($P \geq 0.05$). ALT improved follicular growth and the homogeneity of FS without affecting ovulation.

Key words: Altrenogest, follicular development, ovulation, periweaning, sow

1. Introduction

In pigs, folliculogenesis determines embryogenesis by influencing oocyte quality (1). Larger and more mature follicles generated oocytes of better quality (2,3). In general, selected large follicles at estrus are heterogeneous in size (6.50–10.00 mm) (4), morphology, and hormonal status (5), which reflects the further developmental potential of embryos (1,6).

Altrenogest (ALT; a synthetic progestin) treatment in sows during the weaning period improved follicular development by increasing follicular size at the beginning of the follicular phase and the preovulatory period. Moreover, it improves reproductive performance, including a greater ovulation rate (7), better estrus synchronization (8,9), and a higher embryo survival rate (10).

Even though previous studies revealed improved fertility of the sows that were treated with ALT was a consequence of better follicular development, especially increasing follicular size (11–13), the effect of ALT on the homogeneity of preovulatory follicles was not mentioned. Since follicular diversity is associated with the diversity of embryonic development and survival during early pregnancy (1,6), there is a need to investigate homogeneity of preovulatory follicles for better fertility in sows.

Therefore, the objective of this study was to investigate the effects of ALT treatment during the periweaning period on the homogeneity of follicular development, ovulation rate, and reproductive performance in sows.

2. Materials and methods

The study was conducted on a commercial sow breeding farm housing 5000 sows and using an evaporative cooling system during the rainy season (August to September 2013) in Chiang Mai, Thailand (18°47′25″N, 98°59′04″E).

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2.1. Animals, diets, and housing

Animal manipulations were approved by the Animal Usage and Ethics Committee of Kasetsart University (ACKU 03555). Eighty-seven lactating crossbred sows (Landrace × Large White) that were expected to be replaced after weaning during August 2013 because of old age were used in this study. The parity, total born piglets or litter size (LS), nursing piglets (NP), and piglets weaned per litter (PWL) of sows were 7.55 ± 0.11, 16.41 ± 0.65, 11.87 ± 0.18, and 11.82 ± 0.18, respectively. Moreover, the sows' body condition score (BCS) was assessed (14) at weaning to be between 2.00 and 2.50 in range. The lactation length (LL) of the sows in this study was 25.02 ± 0.21 days.

All sows were fed corn- and soybean-based diets during gestation and lactation. Gestation diets were divided into 2 periods. The feeding level was controlled depending on the sow's BCS. During the 1st–11th weeks of gestation, the diet contained 15.51% crude protein (CP), 3047.53 kcal/kg metabolizable energy (ME), and 0.93% total lysine (Lys) (BCS < 3.00: 2.80 kg/day, BCS = 3.00: 2.40 kg/day, BCS > 3.00: 2.00 kg/day) and during the 12th–15th weeks of gestation, the diet contained 16.41% CP, 3048.18 kcal/kg ME, and 0.94% Lys (BCS ≥ 3.00: 2.80 kg/day, BCS < 3.00: 3.20 kg/day). During lactation, all sows were fed ad libitum with the lactation diet. The lactation diet contained 18.08% CP, 3314.47 kcal/kg ME, and 1.16% Lys. After weaning, they were transferred from the lactation barn to the gestation barn and they were kept in individual gestation stalls. Prior to conception, they were fed ad libitum with the lactation diet twice daily.

Minimum and maximum environmental temperatures were recorded throughout the experiment. The average temperature varied between a minimum of 24.40 ± 0.30 °C and a maximum of 26.60 ± 0.60 °C in the gestation barn and a minimum of 23.20 ± 0.20 °C and a maximum of 26.80 ± 0.50 °C in the lactation barn.

2.2. Altrenogest treatments

The sows were divided into four groups as follows: control, ALT-4–0, ALT-4–2, and 2ALT-4–2. The sows in each group were further divided into 3 slaughter periods for ovarian structural examination: day 1 (DF1) and day 4 (DF4) of the follicular phase, and after the initiation of estrus. Sows were randomly distributed to treatment groups by parity. The control group (n = 20) did not receive ALT (Altresyn®, Ceva Animal Health). For the other groups, the sows were fed a small amount of feed prior to receiving their large meal to get ALT supplementation. ALT was supplemented daily as a top dressing over a small portion of feed in different doses and durations (ALT-4–0: 20 mg/day, for 5 days, D-4–D0, n = 20; ALT-4–2: 20 mg/day, for 7 days, D-4–D2, n = 23; 2ALT-4–2: 20 mg twice a day [40 mg/day]), for 7 days, D-4–D2, n = 24) (D0 = weaning day) (Table 1). ALT was given to the sows in the morning at 0600 hours, except for 2ALT-4–2, which received ALT again at 1800 hours. The main four meals of the day were at 0730, 1230, 1800, and 2000 hours in the lactation barn. The main two meals of the day were at 0700–0730 and 1230–1330 in the gestation barn.

The sows that were slaughtered after estrus detection were checked daily for estrus starting on day 1 after treatment withdrawal (weaning or ALT) using fence-line contact with a mature boar and carried out by trained farm technicians. Estrus was indicated when the sows exhibited a standing heat reflex during a back pressure test in the presence of a boar between 0800–0900 and 1500–1600 hours. The sows were slaughtered 3.48 ± 0.29 days after the initiation of estrus to assess the ovulation rate and ovarian structures. The sows with a delayed return to estrus were monitored for estrus daily until 14 days after treatment withdrawal (weaning or ALT).

2.3. Follicular development

Ovaries were obtained from the slaughtered sows at the specific periods described above and transported to the laboratory in phosphate-buffered saline at 37 °C within 1.50 h (15). The ovaries were examined on the day of slaughter and number of follicles (NF), follicular size in diameter (FS), and ovarian structures (follicle - FL and corpus luteum - CL) (16) were recorded for all animals. The number of CLs revealed the ovulation rate.

Follicles larger than 1 mm were counted. The surface follicular diameter was determined using a caliper. Besides that, FS was categorized into 3 groups; small follicles (<3.00 mm; S), medium follicles (3.00–6.90 mm; M), and large follicles (≥7.00 mm; L). Follicles that were equal to 15.00 mm or larger were considered as large cystic follicles (17,18). The sows that had large cystic follicles were not included in this analysis.

2.4. Statistical analyses

Normality of variables was checked by using the Shapiro–Wilk test. NF, mean FS, and coefficient of variation (CV) of FS (the ratio of standard deviation of FS to mean of FS expressed in percentage values) were analyzed by analysis of covariance (ANCOVA) by using the GLM procedure (SPSS 18, SPSS Inc., Chicago, IL, USA). Model 1 included the effects of treatment, day of the follicular phase, and the interaction between these two factors. In addition, parity, BCS (19), LL (20), and NP (21) were considered as cofactors in this model. When the treatment, day, or interaction effect was significant, the Bonferroni method was used for further multiple comparison tests and simple effects analysis.

The number of CLs or ovulation rate was analyzed by using ANCOVA that included terms for treatment, day of

Table 1. Altrenogest (ALT) treatments in sows and slaughter periods.

Group[1]	n	Day after weaning (D)																			
		- 4	- 3	- 2	- 1	0	1	2	3	4	5	6	7	8	9	10	11	12	13	14	15	16
Control	20						×			×												
							(n = 9)			(n = 5)												
							Heat detection (n = 6)															
ALT-4–0	20			ALT			×			×												
							(n = 6)			(n = 7)												
							Heat detection (n = 7)															
ALT-4–2	23				ALT				×			×										
									(n = 7)			(n = 8)										
									Heat detection (n = 8)													
2ALT-4–2	24				2ALT				×			×										
									(n = 8)			(n = 8)										
									Heat detection (n = 8)													

[1] Group: Control = no ALT treatment; ALT-4–0 = ALT 20 mg/day, 5 days, D-4–D0; ALT-4–2 = ALT 20 mg/day, 7 days, D-4–D2; 2ALT-4–2 = ALT 20 mg twice a day (40 mg/day), 7 days, D-4–D2).

ALT = Altrenogest treatment at 20 mg once a day.

2ALT = Altrenogest treatment at 20 mg twice a day (12-h interval).

D0 = Weaning day.

× = The sows in each group were slaughtered on day 1 (DF1) and day 4 (DF4) of the follicular phase and after the initiation of estrus. Day of follicular phase began 1 day after weaning in the control group and 1 day after ALT withdrawal in the ALT-4–0, ALT-4–2, and 2ALT-4–2 groups. The numbers in parentheses represent the number of sows slaughtered in the different periods.

Heat detection = The duration of estrus detection presented with gray color. The sows were slaughtered after the initiation of estrus.

the follicular phase, and the interaction between these two factors (Model 2). Cofactors including parity, BCS, LL, NP, NF, and days to slaughter (DTS) (the interval between the initiation of estrus was detected and slaughtered day) (4,22) were accounted for in Model 2. The Bonferroni method was used for further multiple comparisons and simple effects analysis when the treatment effect, day effect, or interact effect was significant.

The treatment withdrawal (weaning or ALT) to estrus interval comparison between the groups was analyzed by ANCOVA using the GLM procedure (Model 3). Model 3 included the effects of treatment and cofactors such as parity, BCS, LL, and NP. When the treatment effect was significant, means of the four groups were further analyzed by Bonferroni method.

Comparisons of the percentage of estrus expression between the groups within 7 days and 14 days after treatment (weaning or ALT) withdrawal were evaluated by using the Fisher–Freeman–Halton exact test (23) and Bonferroni corrected pairwise technique (24).

The significance level was considered as $P < 0.05$. The data in the text and tables are presented as mean ± SEM.

3. Results

A sow in the control group, two sows in ALT-4–0, and one sow in ALT-4–2 had large cystic follicles. Therefore, they were excluded from this study.

The cofactors including parity, BCS, LL, NP, and DTS were not significantly different between groups (Table 2).

3.1. Number of follicles (NF), follicular size (FS), and variation of follicular size

NF and FS changed throughout the process of follicular development. The reduction of NF was noticeable on DF4 when compared to DF1 ($P < 0.001$) after treatment withdrawal, although there were no differences between groups ($P \geq 0.05$) (Table 3). While FS was affected by the interaction between groups and days of the follicular phase ($P < 0.001$) (Table 3), FS was larger on DF4 compared to DF1 ($P < 0.05$). On DF1, FS was larger ($P < 0.05$) in the control group compared to the ALT-4–0, ALT-4–2, and 2ALT-4–2 groups. On DF4, FS in the ALT-4–2 and 2ALT-4–2 groups was larger ($P < 0.05$) than in the control and ALT-4–0 groups, and the ALT-4–2 group had the greatest FS (Table 3).

Table 2. Comparisons of covariates between groups.

Covariate	Group[1]	n	Mean ± SEM	Significance
Parity	Control	22	7.64 ± 0.15	NS
	ALT-4–0	19	7.26 ± 0.15	
	ALT-4–2	23	7.61 ± 0.17	
	2ALT-4–2	24	7.92 ± 0.16	
Body condition scores (BCS)	Control	22	2.52 ± 0.06	NS
	ALT-4–0	19	2.68 ± 0.07	
	ALT-4–2	23	2.57 ± 0.08	
	2ALT-4–2	24	2.42 ± 0.07	
Lactation length (LL)	Control	22	24.73 ± 0.16	NS
	ALT-4–0	18	24.56 ± 0.42	
	ALT-4–2	23	25.04 ± 0.35	
	2ALT-4–2	24	25.50 ± 0.12	
Nursing piglets (NP)	Control	21	11.38 ± 0.27	NS
	ALT-4–0	19	11.47 ± 0.38	
	ALT-4–2	23	11.91 ± 0.31	
	2ALT-4–2	24	11.63 ± 0.38	
Days to slaughter (DTS)	Control	6	3.50 ± 0.22	NS
	ALT-4–0	7	3.14 ± 0.14	
	ALT-4–2	8	3.00 ± 0.27	
	2ALT-4–2	8	4.88 ± 0.90	

[1] Group: Control = no ALT treatment; ALT-4–0 = ALT 20 mg/day, 5 days, D-4–D0; ALT-4–2 = ALT 20 mg/day, 7 days, D-4–D2; 2ALT-4–2 = ALT 20 mg twice a day (40 mg/day), 7 days, D-4–D2).
NS = Not significant (P ≥ 0.05).

Table 3. Number of follicles (NF), follicular size (FS), and coefficient of variation of follicular size (CV) among groups and between days within group (mean ± SEM).

Variables	Day[2]	Group[1]								Cofactors				Significance		
		Control		ALT-4–0		ALT-4–2		2ALT-4–2		Parity	BCS	LL	NP	Group	Day	Group × day
		n	Mean ± SEM	n	Mean ± SEM	n	Mean ± SEM	n	Mean ± SEM							
Number of follicles	DF1	8	85.25 ± 7.19 ^{x}	6	98.17 ± 6.90 ^{x}	7	86.29 ± 8.3 ^{x}	8	85.00 ± 7.13 ^{x}	NS	NS	NS	NS	NS	***	NS
(NF)	DF4	5	60.80 ± 5.03 ^{y}	6	80.50 ± 13.83 ^{y}	7	45.00 ± 8.50 ^{y}	8	59.50 ± 5.19 ^{y}							
Follicular size	DF1	8	3.86 ± 0.07 ^{ax}	6	3.39 ± 0.05 ^{bx}	7	3.41 ± 0.08 ^{bx}	8	3.10 ± 0.06 ^{cx}	NS	*	***	NS	***	***	***
(FS, mm)	DF4	5	4.61 ± 0.13 ^{ay}	6	4.30 ± 0.08 ^{ay}	7	6.30 ± 0.14 ^{by}	8	5.20 ± 0.11 ^{cy}							
CV (%)	DF1	8	39.74 ± 4.10	6	36.91 ± 2.45	7	45.01 ± 4.39 ^{x}	8	43.07 ± 3.79	NS	NS	*	NS	NS	**	**
	DF4	5	42.01 ± 5.24 ^{a}	6	39.97 ± 3.34 ^{ab}	7	24.81 ± 3.96 ^{by}	8	40.55 ± 5.27 ^{a}							
	DF1	8	15.15 ± 10.00 ^{abx}	6	0.55 ± 0.25 ^{ax}	7	17.79 ± 13.83 ^{bx}	8	1.43 ± 0.74 ^{abx}	NS	NS	NS	NS	*	***	NS
L follicles (%)	DF4	5	28.18 ± 11.27 ^{aby}	6	16.63 ± 5.54 ^{ay}	7	66.85 ± 12.75 ^{by}	8	37.54 ± 9.48 ^{aby}							
	DF1	8	56.55 ± 6.82 ^{ab}	6	74.52 ± 2.91 ^{a}	7	49.22 ± 8.35 ^{b}	8	56.27 ± 8.02 ^{ab}	NS	NS	NS	NS	**	NS	NS
M follicles (%)	DF4	5	53.83 ± 9.01 ^{ab}	6	66.64 ± 6.13 ^{a}	7	26.74 ± 9.72 ^{b}	8	46.96 ± 8.09 ^{ab}							
	DF1	8	28.30 ± 5.36 ^{x}	6	24.93 ± 2.93 ^{x}	7	32.99 ± 6.18 ^{x}	8	42.30 ± 8.11 ^{x}	NS	NS	NS	NS	NS	***	NS
S follicles (%)	DF4	5	17.99 ± 7.98 ^{y}	6	16.72 ± 3.85 ^{y}	7	6.41 ± 3.23 ^{y}	8	15.51 ± 5.64 ^{y}							

[1] Group: Control = no ALT treatment; ALT-4–0 = ALT 20 mg/day, 5 days, D-4–D0; ALT-4–2 = ALT 20 mg/day, 7 days, D-4–D2; 2ALT-4–2 = ALT 20 mg twice a day (40 mg/day), 7 days, D-4–D2).
[2] Day: DF1 = day 1 of the follicular phase, DF4 = day 4 of the follicular phase.
Cofactors: BCS = body condition score; LL = lactation length; NP = number of nursing piglets.
L follicles (≥7.0 mm), M follicles (3.0–6.9 mm), and S follicles (< 3.0 mm).
$^{a, b, c}$ Values differed significantly among groups (P < 0.05).
$^{x, y}$ Values differed significantly between days within group (P < 0.05).
NS = Not significant (P ≥ 0.05), * = P < 0.05, ** = P < 0.01, *** = P < 0.001.

The variation of follicular size (CV) was influenced by interaction between groups and days ($P < 0.01$). It was similar among groups on DF1**.** However, on DF4, the ALT-4–2 group had less variation ($P < 0.05$) than the control and 2ALT-4–2 groups. Moreover, in comparison between DF1 and DF4 within the same group, the variation of FS did not change, except for the ALT-4–2 group, which had reduced CV ($P < 0.05$) when compared to DF1 (Table 3).

3.2. The percentage of follicles classified by size

The percentage of L follicles increased from DF1 to DF4 in all groups but varied between groups. The ALT-4–2 group had a higher percentage of L follicles but it was not significantly different from the control and 2ALT-4–2 groups. However, it was higher ($P < 0.05$) than that of ALT-4–0 group.

There was no effect of days on treatment, but an effect of groups was detected on the difference in the percentage of M follicles. The lowest percentage of M follicles was noticeable in the ALT-4–2 group, but it did not differ from the control and 2ALT-4–2. The ALT-4–0 group had the highest percentage of M follicles.

The percentage of S follicles on DF4 was lower when compared to DF1. However, it did not vary among groups (Table 3).

3.3. Ovulation rate

After estrus expression, the numbers of CLs, which indicated the ovulation rate, were similar among the four groups (Table 4).

3.4. Treatment withdrawal (weaning or ALT) to estrus interval (TWEI) and percentage of estrus within 7 days and 14 days following treatment (weaning or ALT) withdrawal

ALT treatment affected TWEI ($P < 0.001$). ALT treatment twice a day (2ALT-4–2) extended TWEI, which was longer ($P < 0.05$) than that of the control, ALT-4–0, and ALT-4–2. Moreover, ALT treatment for 7 days postponed TWEI of the sows in ALT-4–2 when compared to the control. TWEI was not different between the control and ALT-4–0 (Table 5).

ALT treatment twice a day (2ALT-4–2) decreased ($P < 0.05$) the percentage of estrus within 7 days after treatment (ALT) withdrawal when compared to the control and ALT-4–0, but it was similar to ALT-4–2. However, all sows were detected to be in estrus by 14 days following treatment withdrawal (Table 6).

4. Discussion

The greater advance of follicular growth decreased NF on DF4 when compared to DF1. The reason for this could be the atresia of follicles during development. During follicular development, FSH is responsible for the growth of small and medium follicles on the ovary surface (recruitment) (25). After that, LH will promote further growth of these follicles to preovulatory size (selection) (25). In the current study, some of the developing follicles were in atresia, probably due to the changes of hormonal status and the level of LH receptors (26). Atresia of follicles occurs in both the recruitment and selection processes (26). After ALT treatment, the growing of medium-sized follicles may generate inhibin dimers, which are specific for FSH inhibition (26). Furthermore, when these follicles develop to larger than 6 mm, they contribute to a higher level of peripheral 17β-estradiol, which has a negative effect on the hypothalamus; this subsequently reduces the concentrations of GnRH, LH, and FSH (26). Therefore, some of the small and medium follicles that need FSH for growth and have insufficient LH receptors will be in atresia (27).

ALT treatment could delay follicular growth. The suppressive effect on follicular growth depended on the dose and duration of ALT treatment. Besides that, during

Table 4. Comparisons of ovulation rate (based on the number of CLs) after estrus expression between groups (mean ± SEM).

Group[1]	n	Ovulation rate	Cofactors						Significance
			Parity	BCS	DTS	LL	NP	NF	
Control	6	23.17 ± 2.17	NS	NS	NS	NS	NS	NS	NS
ALT-4–0	6	27.67 ± 2.97							
ALT-4–2	8	30.25 ± 1.66							
2ALT-4–2	8	26.38 ± 4.62							

[1] Group: Control = no ALT treatment; ALT-4–0 = ALT 20 mg/day, 5 days, D-4–D0; ALT-4–2 = ALT 20 mg/day, 7 days, D-4–D2; 2ALT-4–2 = ALT 20 mg twice a day (40 mg/day), 7 days, D-4–D2).
Cofactors: BCS = body condition score; DTS = days to slaughter; LL = lactation length; NP = number of nursing piglets; NF = number of follicles.
NS = not significant ($P \geq 0.05$).

Table 5. The difference of treatment withdrawal (weaning or ALT) to estrus interval (TWEI) among groups (mean ± SEM).

Group[1]	n	TWEI (days)	Cofactors				Significance
			Parity	BCS	LL	NP	
Control	6	5.00 ± 0.00 [a]	NS	NS	NS	NS	***
ALT-4–0	6	6.00 ± 0.00 [ab]					
ALT-4–2	8	7.25 ± 0.25 [b]					
2ALT-4–2	8	8.88 ± 0.52 [c]					

[1] Group: Control = no ALT treatment; ALT-4–0 = ALT 20 mg/day, 5 days, D-4–D0; ALT-4–2 = ALT 20 mg/day, 7 days, D-4–D2; 2ALT-4–2 = ALT 20 mg twice a day (40 mg/day), 7 days, D-4–D2).
Cofactors: BCS = body condition score; LL = lactation length; NP = number of nursing piglets.
[a, b, c] Values differed significantly among groups ($P < 0.05$).
NS = Not significant ($P \geq 0.05$), *** = $P < 0.001$.

Table 6. The percentage of estrus (%) within 7 days and 14 days after treatment withdrawal (weaning or ALT).

Day[2]	Group[1]								Significance
	Control		ALT-4–0		ALT-4–2		2ALT-4–2		
	n	%	n	%	n	%	n	%	
7	6/6	100.00 [a]	7/7	100.00 [a]	5/8	62.50 [ab]	1/8	12.50 [b]	***
14	6/6	100.00	7/7	100.00	8/8	100.00	8/8	100.00	NS

[1] Group: Control = no ALT treatment; ALT-4–0 = ALT 20 mg/day, 5 days, D-4–D0; ALT-4–2 = ALT 20 mg/day, 7 days, D-4–D2; 2ALT-4–2 = ALT 20 mg twice a day (40 mg/day), 7 days, D-4–D2).
[2] Day: days after treatment (weaning or ALT) withdrawal.
[a, b] Values differed significantly among groups ($P < 0.05$).
NS = Not significant ($P \geq 0.05$), *** = $P < 0.001$.

ALT treatment until after weaning, it may prevent ovulation. Therefore, an accumulation of large-sized follicles without ovulation led to a larger FS of follicles on DF4 in ALT-4–2 and 2ALT-4–2 when compared to the control and ALT-4–0 groups. Further evidence was the increasing percentage of large follicles on DF4 in both groups. The slow growth of follicles in this study agreed with a previous report that, during ALT treatment, the suppressive effect of ALT on LH pulse sustained the follicular development of larger follicles up to a size of approximately 5 mm but did not promote final development to preovulatory size (28). However, a different aspect was found, which was that ALT treatment at 20 mg/day for 7 days did not obstruct follicular development to a large size completely, so large follicles on DF1 in ALT-4–2 were present. Administration of ALT at 20 mg/day allowed further growth of follicles because the period of 12 h after ALT treatment had more variability of LH pulse and concentration that could permit follicular development (28). Meanwhile, ALT treatment at 40 mg/day (20 mg twice a day, 12-h interval; 2ALT-4–2) could inhibit or delay follicular growth completely. Thus, the average FS of ALT-4–2 was larger than that of the 2ALT-4–2 group on DF4. Moreover, the duration of the ALT treatment influenced FS; a short duration of ALT treatment (5 days, ALT-4–0) allowed less time for follicular growth than the long duration (ALT-4–2 and 2ALT-4–2 groups). In addition, several previous studies mentioned that ALT treatment produced larger FS at the beginning of the follicular phase than in the control (11,12). The period of ALT treatment may be involved in this disparity. The shorter period of ALT treatment during lactation and the longer period of ALT treatment after weaning in the previous studies, such as D-1–D7, D-1–D14 (11) and D-1–D6, D-3–D6 (12), allowed a longer period for follicular growth without the suppressive effect from suckling, which inhibited the GnRH pulse generator; subsequently,

LH concentration and LH pulsatility was decreased (29). It was demonstrated in this study that the period of ALT treatment was very important for FS at the beginning of the follicular phase.

Heterogeneity of FS is always present in both gilts and sows (30). The follicular diameter of the ovulatory population varies in size by at least 2–3 mm (31). However, inhibiting ovulation during ALT treatment (32) improved the homogeneity of follicles (CV) of the ALT-4–2 group compared to the control group. Increasing FS without ovulation could generate a pool of similarly sized preovulatory follicles by promoting the later smaller follicles to grow to the same size as the former larger size follicles. Nevertheless, the dose and duration of ALT treatment specified the CV of FS by determining the growing rate of smaller follicles to larger size follicles. The greater intensity of ALT suppression of the 2ALT-4–2 group delayed follicular growth to reach larger size more so than in ALT-4–2; therefore, the CV of the 2ALT-4–2 group was higher than that of the ALT-4–2 group. A short duration of ALT treatment (ALT-4–0) did not provide enough time for small follicles to develop to large follicles, so the CV of the ALT-4–0 group did not reduce between DF1 and DF4. Similarly, in the control sows, the heterogeneity of developing follicles still remained and it was unchanged between DF1 and DF4. Therefore, the proper dose and duration of ALT treatment (ALT-4–2) could generate more homogeneous follicles before ovulation.

ALT treatment did not affect ovulation rate after withdrawal. The ovulation rate of the sows after showing estrous signs was not different among groups. This result confirmed a previous study finding that ALT treatment once a day (15–20 mg/day) did not affect ovulation rate when the lactating period was in the range of 18–24 days (11). In the present study, the lactating period varied between 24.73 and 25.50 days. In contrast to early weaned sows (lactating period equal to 12 days), ALT treatment (20 mg/day) after weaning from D13 to D24 could improve the ovulation rate (7).

The dose and duration of ALT treatment affected TWEI and the percentage of estrus expression. Higher dosages and longer periods of ALT treatment resulted in longer TWEI and lower percentage of estrus within 7 days after treatment withdrawal. This effect might possibly be related to LH secretion. ALT treatment decreased GnRH release and reduced LH and FSH secretion (33), and it postponed weaning to estrus in sows (9). Likewise, ALT, which is a synthetic progesterone, might compromise the frequency of LH pulses, similar to the effect of a negative energy balance that prolonged the weaning to estrus interval (34,35). High levels of progesterone (or ALT) possibly inhibited the signal for the initiation of follicular recruitment (25). For these reasons, TWEI was lengthened in ALT-treated sows when compared to the control sows. This result was similar to a previous report that ALT treatment for 7 days (D-2–D5) postponed estrus expression to 7.40 ± 0.10 days after withdrawal (10). However, the suppressive effect of ALT on LH pulse was reversible because all ALT-treated sows could express estrous signs within 14 days after withdrawal.

In conclusion, a proper dose and duration of ALT treatment (20 mg/day for 7 days; ALT-4–2) could improve FS and decrease the CV of FS without a negative effect on ovulation rate after withdrawal. ALT-4–2 supplementation during the periweaning period in sows might increase sow reproductive performance by improving follicular growth and homogeneity.

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