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Citation	Japanese Journal of Veterinary Research, 65(1), 45-50
Issue Date	2017-02
DOI	10.14943/jjvr.65.1.45
Doc URL	<a href="http://hdl.handle.net/2115/64785">http://hdl.handle.net/2115/64785</a>
Type	bulletin (article)
File Information	65-1_045-050.pdf



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# Ameliorative effects of exogenous gonadotropins on reproductive profiles of replacement gilts with delayed puberty in a farm in Thailand

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Received for publication, July 20, 2016; accepted, December 12, 2016

## Abstract

This study was to investigate the effect of gonadotropins on reproductive profiles of replacement gilts with delayed puberty. Totally, 136 Landrace × Yorkshire crossbred gilts, were categorized into control (n = 58) and treatment (n = 78) groups. Gonadotropins (400 U eCG plus 200 IU hCG) were administered in treatment group only. The results revealed that gilts in treatment group had higher number of gilts with estrus (92.3 vs 25.9%,  $P < 0.001$ ), shorter onset to estrus ( $4.7 \pm 0.3$  vs  $9.0 \pm 0.8$  d,  $P < 0.001$ ), higher number of dominant follicles ( $18.0 \pm 0.2$  vs  $13.2 \pm 0.3$  follicles,  $P < 0.001$ ), and higher farrowing rate (87.5 vs 53.3%,  $P = 0.002$ ) than those in control group. In conclusion, gonadotropins containing 400 IU eCG plus 200 IU hCG could improve reproductive profiles in replacement gilts with delayed puberty.

Key Words: delayed puberty, gilts, gonadotropins

Puberty in the replacement gilts is characterized when the first estrus expression and the first ovulation take place<sup>8)</sup>. However, an exact time of ovulation, two-thirds of standing heat period<sup>21)</sup>, is difficult to be visually investigated; therefore, age at first observed estrus is used to clinically describe age at puberty in the female pigs<sup>24)</sup>. Major factors dominating puberty attainment in the female pigs include animal (e.g., age, body weight, backfat thickness) and managerial (e.g., nutrition, housing, boar contact) factors potentiating via endocrine-reproductive axis<sup>8)</sup>. The replacement gilts, in Thailand, reach

puberty at approximately 200 days of age<sup>19)</sup>. The gilts attaining puberty late can possess a poor number of lifetime reproductive performances<sup>14)</sup> and are removed from the herds earlier than they should be<sup>18)</sup>.

Annually, approximately 50% of the female pigs are removed from the herds due to planned and unplanned reasons<sup>18)</sup>, contributing to the requirement of high number of replacement gilts for an adequate substitution. Furthermore, the replacement gilts in preparatory period are also removed; one-third of the culling reasons are reproductive disorders. Moreover, the most

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doi: 10.14943/jjvr.65.1.45

outstanding reproductive reasons for culling the replacement gilts include anestrus (44.0%), abnormal vaginal discharge (20.5%), repeated breeding (15.5%), not being pregnant (10.0%), and miscellaneous causes (10.0%)<sup>23</sup>.

According to the high substitution rate by replacement gilts in the breeding herds<sup>6</sup>, reproductive performances of the gilts considerably impact the herd productivity. If the number of gilts with delayed puberty increase, it will result in serious problems in terms of the gainfulness and herd managements<sup>22</sup>. In pigs, hormonal application is one of the trustworthy approaches to induce estrus and forbid non-infectious infertility<sup>3</sup>. Equine chorionic gonadotropin (eCG) and human chorionic gonadotropin (hCG) have been used for stimulating follicular growth, ovulation, and estrus expression in swine<sup>13</sup>. Nevertheless, varied results from the administration of those hormones have been observed, especially in prepubertal gilts<sup>2,13</sup>.

Gonadotropins comprising 400 IU eCG plus 200 IU hCG have been one of the outstanding hormones to induce estrus in pigs and authorized to use for reproduction control in swine in the United State of America<sup>7</sup>. These benefit the producers in terms of organizing the replacement gilts into the breeding herds and reducing the costs concerning with non-productive days, feed, and labor<sup>13</sup>. Nevertheless, the study on gonadotropins administration in the gilts with delayed puberty in Thailand has been scant. The present study, thereby, aimed to investigate the effects of exogenous gonadotropins on reproductive profiles of the gilts with delayed puberty problem in a farm in Thailand.

The current study was conducted in a swine commercial herd in eastern region of Thailand. In this farm, all gilts were contacted with mature boars for estrus stimulation at approximately 165 days of age. In total, 136 Landrace × Yorkshire crossbred replacement gilts with body weight  $\geq 120$  kg (range 120–128 kg) and which never expressed estrus before 200 days of age were included in the study. They were

accommodated in an open housing system with a density of 2.0 m<sup>2</sup> per head. Feed was provided by 3 kg/pig/day, meanwhile water was ad libitum accessed from water nipples equipped on the pen. Health status was routinely checked up by experienced veterinarians. The vaccinations were conducted to protect against porcine Parvovirus, classical swine fever, and Aujeszky's disease. Besides, an immunity for porcine reproductive and respiratory syndrome was activated by clinging the replacement gilts with weaned sows culled from the production cycle. In the present study, the animal intervention was approved by the Institutional Animal Care and Use Committee, Mahasarakham University (IACUC-MSU) according to the approval number 002/2016.

The gilts older than 200 days and heavier than 120 kg were checked for backfat thickness and ovarian components on the day of weight measurement. Backfat thickness was individually measured by an A-mode ultrasonography (Renco lean meter<sup>®</sup>, Minneapolis, MN, USA) with ultrasonic probe. It was applied on both sides of the P2 position: 6–8 cm away from the dorsal midline at the last rib level<sup>20</sup>. An average value from both sides of P2 position was regarded as a backfat thickness of individual gilts<sup>24</sup>. Ovarian components were individually performed to confirm that they were pubertal-delayed gilts by real-time ultrasonography (HS-2000, Honda Electronics Co., LTD, Tokyo, Japan) with a 5-MHz convex probe. Once the sonogram did not show any corpus luteum (CL) on both ovaries, hormone treatment was administered in the same day.

Due to hormone administration, they were classified into control (n = 58) and treatment (n = 78) groups. Then, gilts in the treatment group were individually administered with gonadotropins containing 400 IU eCG and 200 IU hCG (Fertipig<sup>®</sup>, CEVA Animal Health, Thailand) via intramuscular route, whereas those in control group were not injected with any substance. This was considered D0 of the experiment.

From two days passed after treatment, estrus

**Table 1. Descriptive statistics (Mean  $\pm$  SEM) of the 136 replacement gilts categorized into control (n = 58) and treatment (n = 78) groups**

Variables	Control (n = 58)	Treatment (n = 78)
Age (d)	207.6 $\pm$ 0.7	209.9 $\pm$ 0.6
Body weight (kg)	125.0 $\pm$ 0.4	124.8 $\pm$ 0.3
Backfat depth (mm)	17.4 $\pm$ 0.2	17.3 $\pm$ 0.2

**Table 2. Reproductive profiles (Mean  $\pm$  SEM) of the pubertal-delayed gilts in control and treatment groups**

Variables	Group of gilts		P value
	Control	Treatment	
Sample number (n)	58	78	-
Number of gilts with estrus (%)	25.9	92.3	$P < 0.001$
Onset of estrus expression (d)	9.0 $\pm$ 0.8	4.7 $\pm$ 0.3	$P < 0.001$
Dominant follicle number	13.2 $\pm$ 0.3	18.0 $\pm$ 0.2	$P < 0.001$
Farrowing rate (%)	53.3	87.5	$P = 0.002$
Total piglets born (head)	9.5 $\pm$ 0.3	10.2 $\pm$ 0.2	$P = 0.237$
Piglet birth weight (kg)	1.1 $\pm$ 0.1	1.2 $\pm$ 0.2	$P = 0.057$

symptoms: standing reflex with vulvar changes (e.g., red, swollen, discharging) were checked in all gilts by back pressure test, together with the presence of high-libido mature boars for two times (morning and evening) on a daily basis for 14 days. Those showed estrus were recorded and examined both ovaries again in order to enumerate the number of dominant follicles. The ovarian follicles with an ultrasonographic diameter of  $>6.0$  mm were considered dominant follicles<sup>13</sup>. Moreover, the gilts showing estrus were mated via conventional artificial insemination (AI) on the day of showing first estrus and were received AI at least two times. The pregnant gilts were kept in individual gestating crates and transferred to farrowing pens prior to the expected farrowing date for seven days. Subsequently, farrowing rate and litter size at birth were further investigated.

All data were manipulated and analyzed statistically via SAS version 9.3 (SAS Inst., Cary, NC, USA). General information, including age, body weight, and backfat thickness were shown as mean  $\pm$  SEM. Reproductive information, including onset of estrus expression, the number of dominant follicles, the number of total piglets

born per litter, and piglet birth weight were presented as mean  $\pm$  SEM. The number of gilts with estrus was presented as percentage of gilts expressing estrus in each group. Farrowing rate was presented as percentage of those transferred to the farrowing pens out of all inseminated gilts. Data comparisons between groups (control vs treatment) were analyzed using Student's t-test except the number of gilts with estrus and farrowing rate using Chi-square test. The significant level was limited at  $P < 0.05$ .

Descriptive statistics of the replacement gilts with delayed puberty problem from control (n = 58) and treatment (n = 78) groups are displayed in Table 1. There were no significant difference on age, body weight, and backfat thickness between both groups ( $P > 0.05$ ).

Reproductive data of the replacement gilts comparing between control and treatment groups are demonstrated in Table 2. Those expressing estrus were 72 out of 78 gilts and 15 out of 58 gilts in treatment and control groups, respectively. This apparently showed that the number of gilts with estrus of treated gilts was significantly higher than that of gilts in control group (92.3 vs

25.9%,  $P < 0.001$ ). Gilts in treatment group expressed first estrus within  $4.7 \pm 0.3$  days, whereas those in control group took averagely  $9.0 \pm 0.8$  days to show first estrus signs ( $P < 0.001$ ). The number of dominant follicles were significantly higher in treated gilts than those in control gilts ( $18.0 \pm 0.2$  vs  $13.2 \pm 0.3$  follicles,  $P < 0.001$ ). After AI, it was found that the farrowing rate of the gonadotropins-treated gilts were higher than that of the control gilts (87.5 vs 53.3%,  $P = 0.002$ ). The total number of piglets born per litter ( $10.2 \pm 0.2$  vs  $9.5 \pm 0.3$  heads,  $P = 0.237$ ) was not significantly different between both groups (Table 2). Besides, the gilts in treatment group tended to produce piglets with higher birth weight than those in control group ( $1.2 \pm 0.2$  vs  $1.1 \pm 0.1$  kg,  $P = 0.057$ ).

The current study demonstrated that the administration of exogenous gonadotropins was beneficial to the gilts with delayed puberty problem. This has been the first study of exogenous gonadotropins application to rectify pubertal delay of the replacement gilts in Thailand as could be seen that the treated gilts possessed significantly higher number of gilts with estrus and lower onset of estrus than those in control group. This indicated that exogenous gonadotropins considerably effected on follicular development in these gilts. The previous studies suggested that FSH effects were indispensable for an initial phase of follicular development<sup>9,12</sup>, as indicated by a number of FSH receptors exclusively expressed on granulosa cells of the follicles smaller than 4 mm in diameter<sup>5</sup>, declined when the follicular diameter was greater than 4 mm, and were absent in larger follicles<sup>15,16</sup>, meanwhile further phases of follicular development until ovulation appeared to be controlled by LH effects<sup>1,4,9,11</sup>. During the follicular development, estrogen was synthesized and stored within the antrum. Finally, estrogen in pre-ovulatory follicles induced standing estrus and estrus characteristics in the female pigs<sup>21</sup>. Considering ovulation, the previous studies reported that ovulation took place only with ovarian follicles  $>5.0$  mm<sup>1,5,16</sup>.

This implied that the gilts in treatment group would have higher ovulation rate than those in control group since the gilts in the treatment group possessed far higher number of dominant follicles than those in control group ( $18.0 \pm 0.2$  vs  $13.2 \pm 0.3$ ,  $P < 0.001$ ). This number corresponded with the previous study, performing gonadotropins treatment in prepubertal gilts (aged 159–174 days), which found that approximately 18 follicles would be ovulating and initiating CLs after treatment, without a significant enhancement in the number or the frequency of cystic follicles or cystic CLs<sup>13</sup>.

It was vividly found that the proportion of gilts showing estrus, in the current study, after gonadotropins injection was significantly higher than those in control group. However, some aspects of application needed to be considered. The former study declared that the administration of exogenous gonadotropins in order to stimulate estrus expression in gilts had effect only in the first two parities since no treatment effects were observed in the pigs in parity number  $\geq 3$ <sup>17</sup>. This reflected that long-term application of exogenous gonadotropins might not be beneficial to induce estrus, especially in the sows with high parity numbers.

In this study, it was found that 15 untreated gilts in control group expressed estrus during the study (within 14 days). This phenomenon could happen as these untreated gilts were about to be in estrus during the period of the study. They responded to other factors activating estrus expression, such as a contact with a high-libido mature boar on a daily basis. These might stimulate estrus expression of the gilts in this group. This was in accordance with the previous study that using boar exposure to stimulate estrus, together with mixing and relocation could induce estrus expression in crossbred gilts within 10 days<sup>10</sup>.

In conclusion, gonadotropins containing 400 IU eCG plus 200 IU hCG could significantly improve the number of gilts with estrus, onset of estrus expression, the number of dominant

follicles, and farrowing rate in the gilts with delayed puberty problem. Consequently, the decision to remove the replacement gilts from the herd due to delayed puberty should be reconsidered since exogenous gonadotropins administration was one of the decent tools to improve their estrus expression and other reproductive profiles.

### Acknowledgments

The authors would like to thank CEVA Animal Health (Thailand) LTD. for the provision of gonadotropins (Fertipig®) in the present study. In addition, the financial support of this study was provided by the research support grant of 2016 annual government statement of expenditure, Mahasarakham University.

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