PMSG Pretreatment Before Fixed-Time Artificial Insemination Improves the Reproductive Performance in Replacement Gilts

Qianqian Zhao
Hebei Agricultural University
Chenyu Tao
Hebei Agricultural University
Qiaoli Wei
Hebei Agricultural University
Zhiwei Zhu
Zhejiang Academy of Agricultural Sciences
Longyan Wang
Hebei Agricultural University
Mingzhi Liu
Hebei Agricultural University
Jing Huang
Zhejiang Academy of Agricultural Sciences
Fuxian Yu
Zhejiang Academy of Agricultural Sciences
Xiaoyu Chen
Zhejiang Academy of Agricultural Sciences
Liang Zhang
Zhejiang Academy of Agricultural Sciences
Junjie Li (lijunjie816@163.com)
College of Animal Science and Technology, Agricultural University of Hebei https://orcid.org/0000-0003-0634-5520
Jianzhi Pan
Zhejiang Academy of Agricultural Sciences

Research

Keywords: PMSG, replacement gilts, FTAI, follicular development, ovulation

DOI: https://doi.org/10.21203/rs.3.rs-88326/v1

Abstract

Background: Fixed-time artificial insemination (FTAI) uses exogenous reproductive hormones to regulate the sexual cycle of sows and realize the mating of pigs at a fixed-time without estrus identification. There is a great difference in litter size following FTAI, which may be due to the ovarian status before treatment. However, the specific underlying mechanism remains unclear.

Results: We selected replacement gilts (n=104) and divided them into four groups: CON (n=35), EST (n=16), pregnant mare serum gonadotrophin (PMSG)-15D (n=26), and PMSG-20D (n=27). The PMSG-15D and PMSG-20D groups were pretreated with PMSG for 15 and 20 days, respectively. The four groups were then subjected to the same FTAI treatment. Pretreatment with PMSG causes ovulation in the gilts, to artificially promote an estrus cycle. At 42 hours after ALT feeding stopped, The follicular diameter of PMSG-15D group (2.94±0.24 mm) was significantly higher ($P<0.05$) than the other groups.and the proportion of gilts with follicular diameter greater than 3 mm in the PMSG-15D group was significantly higher than that in the CON group (37.50% vs 5.88%). The maximum follicular diameter before ovulation in the PMSG-15D group (6.28±0.23 mm) was significantly larger ($P<0.05$) than that of the PMSG-20D group (5.98±0.59 mm) and EST group (5.60±0.47 mm), indicating that the follicular development of the PMSG-15D group was better than that of the other groups. The ovulation time of the PMSG-15D group was concentrated on the 25^{th} to 26^{th} day of FTAI. The ovulation rate was as high as 94.5% in the optimal insemination window period and was more suitable for FTAI. Compared with the CON (52.70±12.71 h) and the PMSG-20D (47.88±13.98 h) groups, the GnRH to ovulation interval (42.33±5.87 h) was significantly shortened in the PMSG-15D group. The level of follicle-stimulating hormone of gilts in the PMSG-15D group was more uniform and significantly lower than that in the CON group ($P<0.01$) at 42 h after Altrenogest feeding. The pregnancy rate (80.77%), total litter size (10.44±2.83), and live litter size (10.00±2.61) of the PMSG-15D group were higher than those of the other groups.

Conclusions: We feasibly optimized FTAI using PMSG pretreatments. When the ovaries of replacement gilts were in the luteal stage owing to PMSG pretreatment for 15 days, the follicular development and reproductive performance of replacement gilts were significantly improved.

Background

With the large-scale development of the pig industry towards batch production, problems, such as high recessive estrus rate, low breeding utilization rate, and inadequate sterilization of piggery, in the traditional breeding modes have become increasingly concerning. The development of new breeding technologies that improve the breeding utilization rate, optimize pig farm management, and reduce labor costs have been the recent focus of research efforts.

Fixed-time artificial insemination (FTAI) regulates the sexual cycle of sows and controls their estrus and ovulation times, using exogenous reproductive hormones, to realize population mating at a fixed-time without estrus identification[1]. The estrus cycle is successfully synchronized after administering 15–

20 mg doses of Altrenogest (ALT) to replacement gilts for 14–18 days[2, 3]. Equine chorionic gonadotrophin (eCG) alone or in combination with Human chorionic gonadotrophin (hCG) stimulates follicular growth and improves estrus and ovulation rates in sows and gilts[4, 5]. Ovulation induction via the gonadorelin (GnRH) analog, buserelin after weaning shortens the interval between weaning and ovulation (WOI) and the interval between estrus and ovulation (EOI) in sows[6]. As a result, FTAI technology effectively improves the estrus and ovulation rates of sows and gilts, shortens the nonproductive days, reduces labor costs, improves production efficiency, and plays an important role in promoting the batch production of the pig industry.

However, the litter size of replacement gilts is significantly different when employing FTAI. A meta-analysis of the effects of ALT on the reproductive performance of gilts showed that the litter size and number of live births significantly improved[7]. Vangroenweghe found that the estrus rate and birth weight of piglets significantly improved after treatment of gilts with ALT and GnRH analogs; however, the litter size was not affected[8]. In a recent study, after discontinuation of ALT treatment, eCG and Porcine luteinizing hormone (pLH) intramuscular injection in gilts resulted in shorter delivery time; however, it resulted in a lower litter size than that of the control group[9].

The prerequisite for successful ovulation induction is the presence of a dominant group of follicles in ovulating ovaries[10]. Previous studies in our laboratory found that under the same FTAI procedure, the estrus rate and pregnancy rate of gilts at the initial stage were all higher than those without puberty, indicating that the complete stage of estrus can promote the estrus, ovulation, and pregnancy of gilts. Therefore, the ovarian state of gilts may greatly influence the FTAI effects. However, this process is resource-intensive and time-consuming during practical application, as it is necessary to select or adjust the ovary status of the replacement gilts before the FTAI procedure. The serum gonadotropin of pregnant equine (PMSG) exerts a dual activity of follicle-stimulating hormone (FSH) and luteinizing hormone (LH) [11]. It also has the most effective effect on the follicular development of replacement gilts at a concentration of 800–1000 IU. PMSG treatment may be an effective way to adjust the ovarian status of replacement gilts[12].

The purpose of this study was to investigate the effect of ovarian state on FTAI. Before feeding the ALT, replacement gilts were injected with 1000 IU PMSG to promote follicular development and ovulation as well as to stimulate the next estrus. After PMSG treatment for varying durations, follicular development varied across groups. We then carried out FTAI and subsequently monitored and analyzed the follicular development, ovulation, and reproductive hormone level changes to lay a foundation for optimizing the FTAI program in replacement gilts.

Methods

Animals and management

This study was conducted in Large White × Landrace crossing breed replacement gilts from the Shuangdingshan Animal Farm (Cicheng, Ningbo, China). Healthy gilts aged 190–220 days (n = 53) and 210–240 days (n = 51) were selected. During the experiment, different groups of gilts were divided into different crates for breeding. All gilts adopted the same feeding and management methods. All were fed a regular diet, and the pig farm was equipped with an independent drinking water nipple to provide free drinking water.

The boar semen used in the mating was purchased from a local boar breeding station, and the breed of the boar was Landrace. Before mating, all the semen was examined, and only semen with sperm motility up to 70%, malformation rate less than 10%, and medium density with microscopic examination were selected. Gilts were examined for pregnancy using ultrasound diagnosis on the 25th day after mating, and gilts that could not be determined for pregnancy were examined for the second time 10 days after the first pregnancy examination.

Experimental design

Our experimental design is depicted in Fig. 1. The replacement gilts of 190–220 days were randomly selected and treated with 1000 IU PMSG for 15 days (n = 26) and 20 days (n = 27), and were divided into two groups, PMSG-15D and PMSG-20D, respectively. FTAI was conducted after 15 or 20 days of PMSG use. Ultrasonic monitoring was conducted at 0–6 days, and blood was collected at 0, 1, 3, and 5 days of PMSG treatment. In addition, replacement gilts aged 210–240 days were randomly divided into two groups, EST (n = 16) group, in which FTAI processing was started for those in estrus and CON (n = 35) group, which was used as a blank control and no processing was done before FTAI programs were performed.

The FTAI procedure is also depicted in Fig. 1. Oral administration of ALT (20 mg per pig) every day for 18 days. 42 h after stopping feeding, the pigs were injected with 1000 IU PMSG, and 80 h later, injected with 100 μg GnRH. Insemination for the first time was conducted 24 h after GnRH injection and the second insemination was conducted 16 h later. The gilts that still had a static reaction at 24 h after the second time were inseminated one more time. On the 20th day of the FTAI (42 h after ALT was stopped), blood was collected. Follicular development of gilts from the four groups was ultrasonically monitored after one to three and 20 days until the end of ovulation fed with ALT. All the hormones used in this study, including ALT (N190403), PMSG (B190707) and GnRH (C190806) were purchased from Ningbo Sansheng Biological Technology Co., Ltd.

Follicular development and ovulation detection

Transabdominal ultrasonography (HS-1600, Honda Electronics Co. Ltd., Toyohashi, Japan) was performed twice a day (8:00 a.m. and 2:30 p.m.) with a 5 MHz multi-convex transducer for follicular development detection on both gilt ovaries. The diameters of the three to seven largest follicles were measured and averaged. Ovulation was presumed to have occurred when previously present pre-ovulatory sized follicles (6–9 mm) disappeared[13]. Follicles larger than 12 mm in diameter and visible for

several consecutive days were defined as cystic follicles[14]. Data from gilts with multiple cyst follicles were excluded from subsequent analyses.

Blood collection and hormone analysis

After the gilts were secured, the blood samples collected from the anterior jugular vein with a 10 mL syringe were transferred to a 5 mL coagulation tube for 30 min. After the serum was precipitated, the blood samples were centrifuged at 3000 rpm for 4 min, and the serum was absorbed into the centrifuge tube and stored in the refrigerator for 20 min for testing.

LH (JM-10117P1), progesterone (P_4, JM-01368P1), and estradiol (E_2, JM-01314P1) analyses were performed using an enzyme-linked immunosorbent assay, (Jingmei Biotechnology Co. Ltd., Yancheng, China). The detection ranges of the assays were 22–800 pmol/L, 8–200 pmol/L, and 20–500 pg/mL, respectively. Blood samples were analyzed for FSH(CSB-E06791P) using a direct commercial kit purchased from Cosmo Bio, Japan. The detection ranges of the assay was 20–700 mIU/ml.

Statistical analyses

Data were analyzed by repeated measures analysis of variance (split-plot ANOVA, Gill & Hafs, 1971) using the general linear model with repeated measures. The SPSS 21.0 statistical software was used for one-way ANOVA with different replicates. Duncan's test was used for multiple comparisons and significance analysis. The estrus rate and pregnancy rate of gilts were measured using the Chi-square test. Except for χ^2test indexes, they were all expressed and calculated as mean ± standard deviation (mean ± SD). All results were considered statistically significant at $P < 0.05$.

Results

Follicular development and hormone levels in replacement gilts preconditioned with PMSG

The follicle development trend of the replacement gilts after PMSG treatment is shown in Fig. 2a. After PMSG treatment, two of the 24 gilts monitored by ultrasound were not in estrus, and thus data of the two gilts were not considered. After PMSG treatment, the average follicular diameter (3.23 mm) exceeded 3 mm on the afternoon of the second day, and the average follicular diameter reached a maximum of 4.16 mm on the morning of the fifth day. It then began to decrease until the average follicular diameter of 2.89 mm dropped to less than 3 mm on the afternoon of the sixth day. The distribution of ovulation time of gilts treated with PMSG is shown in Fig. 2b. The results revealed that 91% of gilts ovulated on the fifth to sixth day after PMSG treatment.

After PMSG pretreatment, the contents of E_2, LH, and FSH in gilts increased with time and reached the highest level on day 5. The contents of E2 and LH in PMSG pretreatment were significantly higher on day 5 than those on day 0, day 1 and day 3($P < 0.05$). However, there was no significant difference in the

contents of FSH (Fig. 3). The content of P_4 did not change significantly within 0–5 days after PMSG treatment ($P > 0.05$), and the concentration fluctuated within 150–200 pg/mL (Fig. 3).

Effect on follicular development and hormone levels in FTAI replacement gilts

The average follicular diameter (2.59 mm) of the CON group and the average follicular diameter (2.62 mm) of the PMSG-15D group were all lower than 3 mm when the FTAI started, namely began to feed ALT. The average follicular diameter of the PMSG-20D group was 3.18 mm, which tended to be that of the EST group (3.83 mm). The average follicular diameter of the gilts in the EST group decreased to 2.98 mm in the afternoon of the second day after FTAI, and the follicular diameter of all the replacement gilts was synchronized to the same level on the third day, as shown in Fig. 4.

After PMSG treatment on the afternoon of the 20th day, the follicles of the four groups of gilts began to develop, and there was a significant trend of follicular enlargement. The average follicular diameters of the four groups of gilts at different time points of D20-PM (PMSG treatment) and D24-PM (the first artificial insemination) was, from the highest to the lowest, PMSG-15D, CON, PMSG-20D, and EST groups. In the afternoon of the 24th day, the follicle diameter of the four groups reached the maximum, which was 6.08 mm, 5.76 mm, 5.72 mm, and 5.00 mm, respectively. After that, the average follicular diameter began to decrease, and on the 26th day, the follicular diameter decreased to less than 3 mm (Fig. 4).

At 42 hours after ALT feeding was stopped, the maximum observed values of preovulation follicular diameter of the gilts in different treatment groups, from the highest to the lowest, were the PMSG-15D, CON, PMSG-20D, and EST groups (Table 1). The follicular diameter of PMSG-15D group was significantly higher than that in CON group (2.94 ± 0.24 mm vs 2.56 ± 0.30, $P < 0.05$), EST group (2.94 ± 0.24 mm vs 2.72 ± 0.44 mm, $P < 0.05$) and PMSG-20D (2.94 ± 0.24 mm vs 2.60 ± 0.24, $P < 0.05$).There was no significant difference between CON group, EST group and PMSG-20D group. At PMSG treatment, the gilts proportion of follicle diameter greater than 3 mm was different in different treatment groups, ranging from the largest to the lowest, were EST group, PMSG-15D group, PMSG-20D group and CON group respectively(Table 1).The proportion of gilts with follicular diameter greater than 3 mm in the PMSG-15D group was significantly higher than that in the CON group (37.50% vs 5.88%), and there was no significant difference among the CON group, the PMSG-15D group and the PMSG-20D group.

Table 1
The follicular diameter at 42 hours after ALT feeding was stopped in different treatment groups

Group	Follicular diameter at 42 hours after ALT feeding was stopped (mean ± SD, mm)	Proportion of gilts with follicular diameter greater than 3 mm (%)
CON	2.56 ± 0.30^{b}(2.14–3.36)	5.88^{b}(1/17)
EST	2.72 ± 0.44^{b}(1.97–3.33)	37.50^{a}(6/16)
PMSG-15D	2.94 ± 0.24^{a}(2.67–3.61)	22.22%(4/18)
PMSG-20D	2.60 ± 0.24^{b}(2.14–3.15)	6.25%(1/16)
$^{a, b}$ means with different letters in the same column and within a category differed (P < 0.05)		

The maximum observed values of preovulation follicular diameter of the gilts in different treatment groups, from the highest to the lowest, were the PMSG-15D, CON, PMSG-20D, and EST groups (Table 2). Preovulation maximum follicular diameter in the PMSG-15D (6.28 ± 0.23 mm vs. 5.60 ± 0.47 mm, P < 0.01), PMSG-20D (6.06 ± 0.38 mm vs. 5.60 ± 0.47 mm, P < 0.01), and CON (5.98 ± 0.59 mm vs. 5.60 ± 0.47 mm, P < 0.05) groups were significantly higher than that of the EST group. Moreover, the preovulation maximum follicular diameter in the PMSG-15D group was significantly higher than that of the PMSG-20D group (6.28 ± 0.23. vs. 5.98 ± 0.59 mm, P < 0.05; Table 2).

Table 2
Follicular diameter before ovulation in different treatment groups

Group	The largest diameter of the follicle before ovulation (mean ± SD, mm)	Variation range of follicular diameter before ovulation (mm)
CON	6.06 ± 0.38^{Ab}	5.27–6.67
EST	5.60 ± 0.47^{Bc}	4.18–6.07
PMSG-15D	6.28 ± 0.23^{Aa}	5.85–6.66
PMSG-20D	5.98 ± 0.59^{b}	5.19–7.58
$^{a, b, c, A, B}$ means with different letters in the same column and within a category differed (P < 0.05)		

At 42 hours after ALT feeding was stopped, hormone levels of E2, LH, and P4 of gilts in the CON, EST, PMSG-15D, and PMSG-20D groups showed no significant difference ($P > 0.05$, Fig. 5a, b, d). Analysis of FSH content showed that the EST, PMSG-15D, and PMSG-20D groups were significantly lower than those of the CON group ($P < 0.01$). Furthermore, the consistency of FSH levels in the PMSG-15D group was higher than that in the EST and PMSG-20D groups (Fig. 5c).

Correlation between estrus and ovulation interval and estrus duration

The ovulation time distributions of the four groups were counted and are shown in Fig. 6. Ovulation time of gilts of the CON, EST, PMSG-15D, and PMSG-20D groups were concentrated on days 23–26, 23–27, 25–26, and 25–27 after ALT feeding, respectively. Moreover, the highest ovulation rates of the four groups all appeared on day 25, and they were 76.5%, 68.8%, 94.5%, and 81.3%, respectively.

The time interval between GnRH injection and ovulation of the PMSG-15D (42.33 ± 5.87 vs 52.70 ± 12.71, $P < 0.05$) and EST (38.13 ± 21.10 vs. 52.70 ± 12.71, $P < 0.01$) groups was significantly lower than that of the CON group. The time interval between GnRH injection and ovulation of the PMSG-20D group (47.88 ± 13.98) was not significantly different from the other three groups ($P > 0.05$, Table 3). The interval between estrus and ovulation (24.78 ± 12.30, $P < 0.01$) and the duration of estrus (41.63 ± 17.45, $P < 0.05$) in the EST group were significantly lower than those in the other three groups. However, there was no significant difference between the CON, PMSG-15D, and PMSG-20D groups ($P > 0.05$, Table 3).

Table 3
Interval of GnRH injection to ovulation, estrus to ovulation, and estrus duration of gilts in different treatment groups

Group	Interval of GnRH injection to ovulation (h)	Interval of estrus to ovulation (h)	Estrus duration (h)
CON	52.70 ± 12.71^{Aa}	44.47 ± 14.71^{B}	56.82 ± 22.46^{b}
EST	38.13 ± 21.10^{B}	24.78 ± 12.30^{A}	41.63 ± 17.45^{a}
PMSG-15D	42.33 ± 5.87^{b}	42.33 ± 12.93^{B}	53.67 ± 15.60^{b}
PMSG-20D	47.88 ± 13.98^{ABb}	43.88 ± 18.16^{B}	55.88 ± 12.53^{b}
$^{a, b, A, B}$ means with different letters in the same column and within a category differed ($P < 0.05$)			

In the CON ($r = 0.71$), PMSG-15D ($r = 0.65$), and PMSG-20D ($r = 0.63$) groups, there was a significant correlation between the interval between estrus and ovulation and the duration of estrus ($P < 0.01$); however, no significant correlation was found in the EST group ($P > 0.05$, Fig. 7).

Effect on reproduction performance in FTAI replacement gilts

The estrus rate of gilts in the EST group (87.50%) was the lowest after the FTAI procedure. Compared with the PMSG-15D group (97.12%), the estrus rate in the EST group was not significant difference ($P > 0.05$), however, it was significantly lower than that of the PMSG-20D (100%) and CON (100%; $P < 0.05$) groups. The analysis of pregnancy rate of gilts in different treatment groups showed that it was highest in the PMSG-15D group (80.77%), which was 8.55%, 12.02%, and 2.99% higher than in the CON (72.22%), EST (68.75%), and PMSG-20D (77.78%) groups, respectively. However, the difference was not significant ($P < 0.05$). The total litter size and live litter size of the PMSG-15D group were the highest (10.44 ± 2.83 and 10.00 ± 2.61) among the four groups, which were 0.49 and 0.57 heads higher than those of the CON group (9.95 ± 2.44 and 9.43 ± 2.60) and live litter size of the PMSG-15D group, respectively. However, the difference was not significant ($P > 0.05$, Table 4).

Table 4
Estrus rate, pregnancy rate, and litter efficiency of the gilts in different treatment groups

Variables	CON	EST	PMSG-15D	PMSG-20D
NO. of assigned	36	16	26	27
NO. of estrus	36	14	25	27
Estrus rate/%	100^{a} (36/36)	87.50^{b} (14/16)	97.12^{ab}(25/26)	100^{a} (27/27)
No. of pregnant	26	11	21	21
Pregnant rate/%	72.22 (26/36)	68.75(11/16)	80.77(21/26)	77.78(21/27)
No. of piglets per litter	9.95 ± 2.44	9.91 ± 2.34	10.44 ± 2.83	10.05 ± 2.25
No. of alive piglets per litter	9.43 ± 2.60	9.09 ± 1.87	10.00 ± 2.61	9.63 ± 2.06
$^{a, b}$ means with different letters in the same column and within a category differed ($P < 0.05$)				

Discussion

PMSG stimulates follicular growth and ovulation[15]. Some experimental studies showed that the estrus rate of gilts injected with 725–1000 IU PMSG was 70–100%[16], whereas those injected with 363–600 IU PMSG was only 25–52%[17, 18]. Therefore, a dose of 1000 IU PMSG was selected for pretreatment in this study. The follicle average diameter of gilts that were pretreated with PMSG before FTAI reached a maximum after five days. At the same time, E2, LH, and FSH also increased sharply on the fifth day, which led to ovulation between 5–6 days after PMSG treatment. Thus, PMSG pretreatment was shown to induce an estrus cycle in gilts and synchronize follicular development and ovulation time preliminarily.

Based on the reproductive cycle of gilts[19], we assumed that the ovaries of gilts were in the luteal phase on the 10th day after ovulation (15–16 days after PMSG pretreatment), and in the follicular phase on the 15th day after ovulation (20–21 days after PMSG pretreatment). Therefore, FTAI treatment was selected after 15 and 20 days of PMSG pretreatment to explore the influence of different ovarian states on the FTAI effect in replacement gilts.

When the FTAI procedure was started, the average size of follicles in the PMSG-15D group was below 3 mm, and no developing or mature follicles were present, indicating that the ovarian state of gilts in the PMSG-15D group was in line with the expectation that the ovarian follicle would be in the luteal phase after 15 days of PMSG treatment. The follicle sizes of the gilts in the PMSG-20D and EST groups were similar and larger than those in the PMSG-15D and CON groups, indicating that developing follicles appeared on the ovaries of gilts in the PMSG-20D group and that the ovarian state in the PMSG-20D group was in the follicular stage.On the third day after ALT treatment, the follicles of the four groups were synchronized to the same level and the diameter of the follicles was less than 3 mm, indicating that ALT played a role in effectively preventing the development of small and medium follicles, to control synchronous estrus[20]. PMSG has follicle-stimulating activity and promotes follicular development[21]. After the PMSG injection, follicular diameter tended to increase with time. GnRH effectively improves LH secretion and promotes the final maturation and ovulation of dominant follicles. After intramuscular injection of GnRH, the follicular diameter of gilts in the four groups increased to the maximum, and the follicle reached maturity. On the second day after mating, the follicular diameter of all gilts dropped below 3 mm again, marking the end of ovulation.

Although the follicular development trend of the four groups was similar, the follicular development level was different at different time points. The study found that PMSG-15D had the largest follicle diameter at the time of PMSG injected compared with other groups. This is consistent with the results of Soede[22]. When gilts were fed with Regumate® (trade name of ATL) for 18 days during the pre-follicular phase, the average follicular size of the treated gilts increased by 0.5 mm compared to the control group, indicating that follicular selection and development had been differentiated after treatment with ALT. There is a continuous growth and atresia of ovarian follicles during days 7 to 15 of the estrous cycle, without evidence of follicular dominance, resulting in a stable size and number of small follicles on the ovaries. Moreover, ALT was found to have less inhibitory effect on follicular growth than endogenous progesterone (with or without ALT)[23, 24]. Thus the application of ALT may not adversely affect the follicles during luteal phase.

After the effects of PMSG and GnRH, the differences in follicular development between the gilts in each group were significantly increased. The maximum follicular diameter before ovulation in the PMSG-15D group was significantly larger than that in the EST group and the PMSG-20D group (Table 1). It can be seen that the gilts in the PMSG-15D group (that is, the initial state is in the luteal phase) undergoing FTAI, showed the best growth ability in terms of the degree of follicular development. Soede found that the size of follicles before ovulation was positively correlated with luteal weight on the fifth day after

ovulation[25].Similarly, a positive correlation was also observed in cows and ewes between the size of the follicle before ovulation and the size of the corpus luteum[26]. In cattle, the oocytes from larger follicles showed faster embryonic developmental ability than those from smaller follicles, which demonstrated the relationship between follicular size and oocyte capacity[27–29]. From these results, it can be inferred that larger follicles may have more capable oocytes, and may form larger or more functional luteal bodies after ovulation, potentially improving the subsequent fertility.

The results showed that the variation range of follicle size in PMSG-15D group at PMSG treatment and pre-ovulation was smaller than that in other groups, and the difference value was only 0.94 mm and 0.81 mm, indicating that the gilts in PMSG-15D group had a better synchronism in follicle development after FTAI treatment. The results also showed that the distribution of ovulation time of gilts in the four groups was different. All the gilts in PMSG-15D group completed ovulation within 36 hours, and the distribution of ovulation time was more concentrated than that in CON group, EST group and PMSG-20D group, indicating that the ovulation synchrony was better in gilts pretreated with PMSG for 15 days. The reason for the difference in follicular development and ovulation time was closely related to the hormone level in gilts. To verify the hormone level change, blood samples were collected at 42 hours after stopping feeding ALT.The level of FSH in the PMSG-15D group was the lowest, and it had the highest uniformity (Fig. 9).This may be due to that FSH of gilts in PMSG-15D group were not only inhibited by exogenous ATL supplementation, but also inhibited by endogenous progesterone secreted in luteal stage[24].Subsequently, follicular development and ovulation in gilts were further improved by PMSG and GnRH treatment.

Successful insemination of gilts depends primarily on the ovulation and inseminationtime[30]. In theory, sperm show optimal fertility at 24 hours after insemination[31, 32]. However, if insemination is done too late, the oocytes would be degraded[33], which negatively impacts the sow fertility, including reductions in pregnancies, fertilization rates[34], and embryo survival[35]. Since the artificial insemination of FTAI was performed in the afternoon and morning on the 24th and 25th day, respectively, the optimal ovulation time was concentrated on the 25th day. This experiment found that the interval between GnRH injection to ovulation of PMSG-15D group was significantly shortened, and the ovulation rate (94.5%) was the highest on the 25th day, indicating that gilts in PMSG-15D group ovulated earlier than the other groups, and were more suitable for the current FTAI procedure. However, we also found that 18.9% of gilts in the PMSG-20D group ovulated after 25 days (Fig. 6), indicating that the ovulation time was too late.

In the EST group, the average follicle diameter was not the largest when treated with PMSG, but the percentage of gilts with an average follicle diameter above 3 mm was the highest. Therefore, the time from follicular development to ovulation in the EST group of gilts did not need to be too long after stoppong feeding of ATL, leading to a significant shorter intrval between GnRH injection to ovulationthan the other groups. However, gilts in the EST group had smaller preovulatory follicles, poor ovulation synchronization, and low reproductive efficiency.The gilts in EST group was in the follicular stage at the beginning of ATL treatment, and the follicles on the ovary were mainly composed of large follicles[23].

Previous study had found a relatively low but constant concentration of LH during ALT treatment may cause the growth of large follicles[36]because large follicles are more sensitive to LH than small follicles[37]. In cows, ALT treatment for synchronization the estrous cycle had been found to prolong the lifespan of the dominant follicle, preventing it from becoming atretic[36]. Thus, if the large follicle was not atresia and degraded during ALT treatment, a portion of the follicle will continue to develop until ovulate after stopping feeding ALT. This may lead to excessive maturation of pig oocytes, which may negatively affect the maturation and fertilization of oocytes[38].

In addition,Almeida found that the ovulation time depends on the duration of estrus, and there was a strong correlation between estrus duration and ovulation interval ($r = 0.57$, $P = 0.0001$)[39]. In this experiment, the CON, PMSG-15D, and PMSG-20D groups all showed a positive correlation between estrus duration and the interval from estrus to ovulation, while only the EST group showed no correlation (Fig. 7). Therefore, although the EST group ovulated earlier, it did not follow the correlation rule and presented with the worst follicular development, poor synchronization of ovulation time, and other factors explained the poor reproductive performance of the gilts in the EST group in this experiment.

Ultrasonic monitoring results of the PMSG-15D group showed that follicular development and ovulation time were superior to those of other groups since the initial state ovary was in the luteal phase. In subsequent FTAI, the reproductive performance of the PMSG-15D group improved, including the pregnancy rate (80.77%) and litter size (10.44 ± 2.83) (Table 3). However, the pregnancy rate and litter size of the PMSG-15D group did not increase significantly. This may be mainly because litter size is a compound variable influenced by many factors, such as follicular mass[26], ovulation number[40], and uterine volume[41]. Hazeleger found that sows with feed restriction had smaller follicles and lower ovulation rates, indicating that follicle diameter before ovulation could reflect the number of ovulations on the ovaries[42]. Furthermore, the ovulation rate is moderately heritable and positively correlated with the litter size of gilts. Therefore, the lack of significant improvement in the litter rate in the PMSG-15D group was likely due to the increased ovulation rate and the embryo crowding caused by the limited uterine volume of gilts[43–45].

Conclusions

We analyzed the follicular development, ovulation, and reproductive performance of replacement gilts in the luteal stage, pretreated with PMSG before undergoing FTAI and found that those pretreated with PMSG for 15 days showed better reproduction performance than those in the control group. It was feasible to optimize the FTAI program with PMSG pretreatment.

Declarations

Authors' contributions

JJL、JZP designed the study and revised manuscript. QQZ performed all the experiments, analyzed the data and wrote the manuscript. CYT participated in the discussion and wrote the manuscript. QLW helped solve the operational problems and data collection of the experiment. MZL and LYW helped collect the samples. ZWZ、JH was involved in the selection of sows. XYC worked on the ELISA experiment. FXY、LZ gives constructive analysis through discussion. All authors read and approved the final manuscript.

Funding

This work was supported by grants from the National Key R&D Program of China (2017YFD0501901), S&T Program of Hebei(20326615D), S&T Program of Zhejiang (2016C02054-6), Zhejiang provincial governor fund (2020R30A60C01) and National Natural Science Foundation of China (31802063) .

Availability of data and materials

The data analyzed during the current study are available from the corresponding author on reasonable request.

Ethics approval

All experiments were performed in accordance with the Guide for the Care and Use of Laboratory Animals prepared by the Institutional Animal Care and Use Committee of Hebei Agricultural University, China.

Consent for publication

Not applicable.

Competing interests

The authors declare that they have no competing interests.

References

1. De Rensis F, Kirkwood R N. Control of estrus and ovulation: Fertility to timed insemination of gilts and sows[J]. Theriogenology. 2016, 86(6): 1460-1466.
2. Stevenson J S, Davis D L. Estrous synchronization and fertility in gilts after 14- or 18-day feeding of altrenogest beginning at estrus or diestrus[J]. Journal of animal science. 1982, 55(1): 119.
3. G M Ravagnani, C H G Martinez, R F Carnevale, at al. Effects of oestrous synchronization with altrenogest in gilts on endometrial and embryonic characteristics[J]. Animal. 2020 Sep;14(9):1899-1905.
4. van Wettere W H E J, Weaver A C, Greenwood E C, et al. Controlling lactation oestrus: The final frontier for breeding herd management[J]. Mol Reprod Dev. 2017.
5. de Jong E, Kauffold J, Engl S, et al. Effect of a GnRH analogue (Maprelin) on the reproductive performance of gilts and sows[J]. Theriogenology. 2013, 80(8): 870-877.

6. Pearodwong P, Tretipskul C, Soede N M, et al. Factors affecting estrus and ovulation time in weaned sows with induced ovulation by GnRH administration in different seasons[J]. Journal of veterinary medical science. 2019, 81(11): 1567-1574.
7. Wang Z, Liu B S, Wang X Y, et al. Effects of altrenogest on reproductive performance of gilts and sows: A meta-analysis[J]. Anim Reprod Sci. 2018, 197(197): 10-21.
8. Vangroenweghe F, Goossens L, Jourquin J. An evaluation of gonadotropin-releasing hormone analogue administered to gilts and sows on subsequent reproductive performance and piglet birth weight[J]. Porcine Health Management. 2016, 2(1): 1-7.
9. Rodrigues L, Amezcua R, Cassar G, et al. Comparison of Single, Fixed-Time Artificial Insemination in Gilts Using Two Different Protocols to Synchronize Ovulation[J]. Animals. 2020, 10(2): 306.
10. Robert V Knox. Recent advancements in the hormonal stimulation of ovulation in swine [J]. Vet Med (Auckl). 2015 Oct 5; 6:309-320.
11. Walker Farmer S, Papkoff H. Immunochemical Studies with Pregnant Mare Serum Gonadotropin[J]. Biology of Reproduction. 1979, 2(21): 425-431.
12. Brüssow K P, Wähner M. Biological and technological background of estrus synchronization and fixed-time ovulation induction in the pig[J]. Biotechnology in Animal Husbandry. 2011, 27(3): 533-545.
13. van Leeuwen J J J, Williams S I, Kemp B, et al. Post-weaning Altrenogest treatment in primiparous sows; the effect of duration and dosage on follicular development and consequences for early pregnancy[J]. Animal Reproduction Science. 2010, 119(3-4): 258-264.
14. Tummaruk P, Kesdangsakonwut S. Factors affecting the incidence of cystic ovaries in replacement gilts[J]. Comparative Clinical Pathology. 2012, 21(1): 1-7.
15. Willis H J, Zak L J, Foxcroft G R. Duration of lactation, endocrine and metabolic state, and fertility of primiparous sows[J]. Journal of Animal Science. 2003, 81(8): 2088-2102.
16. Bolamba D, Matton P, Estrada R, et al. Effects of pregnant mare's serum gonadotropin treatment on follicular populations and ovulation rates in prepuberal gilts with two morphologically different ovarian types[J]. J Anim Sci. 1992, 70(6): 1916-1922.
17. Gama R D, Vianna W L, Pinese M E, et al. Different doses of porcine luteinizing hormone in precocious puberty induction in gilts[J]. Reprod Domest Anim. 2005, 40(5): 433-435.
18. V do Lago, Wl Vianna, Rd Gama, et al. Second oestrus synchronization and precocious embryo viability after puberty induction in gilts by the use of gonadotrophin treatment [J]. Reproduction in Domestic Animals. 2005 Apr; 40(2): 141-4.
19. Soede N M, Langendijk P, Kemp B. Reproductive cycles in pigs[J]. Anim Reprod Sci. 2011, 124(3-4): 251-258.
20. Xiao H, Sun P, Sun F, et al. Pharmacokinetics of altrenogest in gilts[J]. J Vet Pharmacol Ther. 2019, 42(6): 660-664.

21. Kirkwood R N, Kauffold J. Advances in Breeding Management and Use of Ovulation Induction for Fixed-time AI[J]. Reproduction in Domestic Animals. 2015(50): 85-89.
22. Soede N M, Bouwman E G, Langendijk P, et al. Follicle development during luteal phase and altrenogest treatment in pigs[J]. Reprod Domest Anim. 2007, 42(3): 329-332.
23. Sara I Williams, R Luzbel de la Sota. Follicular dynamics and ovulation time in gilts and post-weaning sows [J]. Can Vet J . 2017 Jan;58(1):65-69.
24. Guthrie H D, Grimes R W, Cooper B S, et al. Follicular atresia in pigs: measurement and physiology[J]. Journal of animal science. 1995, 73(9): 2834.
25. Soede N M, Hazeleger W, Kemp B. Follicle size and the process of ovulation in sows as studied with ultrasound[J]. Reproduction in domestic animals. 1998, 33(3-4): 239-244.
26. Bagg M A, Nottle M B, Armstrong D T, et al. Relationship between follicle size and oocyte developmental competence in prepubertal and adult pigs[J]. Reproduction fertility and development. 2007, 19(7): 797.
27. Perry G A, Smith M F, Lucy M C, et al. Relationship between follicle size at insemination and pregnancy success[J]. Proc. Natl. Acad. Sci. U.S.A. 2005, 102(14): 5268-5273.
28. Murdoch W J, Van Kirk E A. Luteal dysfunction in ewes induced to ovulate early in the follicular phase[J]. Endocrinology (Philadelphia). 1998, 139(8): 3480-3484.
29. Salamone D F, Adams G P, Mapletoft R J. Changes in the cumulus-oocyte complex of subordinate follicles relative to follicular wave status in cattle[J]. Theriogenology. 1999, 52(4): 549-561.
30. Waberski D, Weitze K F, Gleumes T, et al. Effect of time of insemination relative to ovulation on fertility with liquid and frozen boar semen[J]. Theriogenology. 1994, 42(5): 831-840.
31. Sumransap P, Tummaruk P, Kunavongkrit A. Sperm distribution in the reproductive tract of sows after intrauterine insemination[J]. Reproduction in Domestic Animals. 2007, 42(2): 113-117.
32. Ulguim R R, Bortolozzo F P, Wentz I, et al. Ovulation and fertility responses for sows receiving once daily boar exposure after weaning and OvuGel® followed by a single fixed time post cervical artificial insemination[J]. Theriogenology. 2018, 105: 27-33.
33. Sarath K K, Pushpakumara A, Alexander B. The effect of pregnant mare serum gonadotrophin (PMSG) and GnRH injection on weaning to oestrus interval and litter size of artificially inseminated sows[J]. Tropical Agricultural Research. 2013, 24 (3): 270-278.
34. Hunter R H. Physiological factors influencing ovulation, fertilization, early embryonic development and establishment of pregnancy in pigs[J]. Br Vet J. 1977, 133(5): 461-470.
35. Knox R V. PHYSIOLOGY AND ENDOCRINOLOGY SYMPOSIUM: Factors influencing follicle development in gilts and sows and management strategies used to regulate growth for control of estrus and ovulation1[J]. Journal of Animal Science. 2019, 97(4): 1433-1445.
36. Redmer D A, Day B N. Ovarian activity and hormonal patterns in gilts fed allyl trenbolone [J]. Journal of animal science. 1981, 53(4): 1088.

37. J Sirois, J E Fortune. Lengthening the bovine estrous cycle with low levels of exogenous progesterone: A model for studying ovarian follicular dominance[J]. Endocrinology. 1990,127 (2): 916-925.
38. Revah I, Butler W R. Prolonged dominance of follicles and reduced viability of bovine oocytes[J]. Journal of Reproduction and Fertility. 1996, 106(1): 39-47.
39. Almeida F R C L, Novak S, Foxcroft G R. The time of ovulation in relation to estrus duration in gilts[J]. Theriogenology. 2000, 53(7): 1389-1396.
40. Knox R V . Recruitment and selection of ovarian follicles for determination of ovulation rate in the pig[J]. Domestic Animal Endocrinology, 2005, 29(2):385-397..
41. Da Silva C L A, Broekhuijse M L W J, Laurenssen B F A, et al. Relationship between ovulation rate and embryonic characteristics in gilts at 35 d of pregnancy[J]. Journal of Animal Science. 2017, 95(7): 3160-3172.
42. Hazeleger W, Soede N M, Kemp B. The effect of feeding strategy during the pre-follicular phase on subsequent follicular development in the pig[J]. Domestic Animal Endocrinology, 2005, 29(2):362-370.
43. Town S C, Patterson J L, Pereira C Z, et al. Embryonic and fetal development in a commercial dam-line genotype[J]. Anim Reprod Sci. 2005, 85(3-4): 301-316.
44. Da S C, Laurenssen B, Knol E F, et al. Validation of transrectal ultrasonography for assessment of corpora lutea characteristics in pregnant sows and its relationship with litter characteristics at birth[J]. Transl Anim Sci. 2017, 1(4): 507-517.
45. Almeida F R, Kirkwood R N, Aherne F X, et al. Consequences of different patterns of feed intake during the estrous cycle in gilts on subsequent fertility[J]. Journal of Animal Science. 2000, 78(6): 1556.

Figures

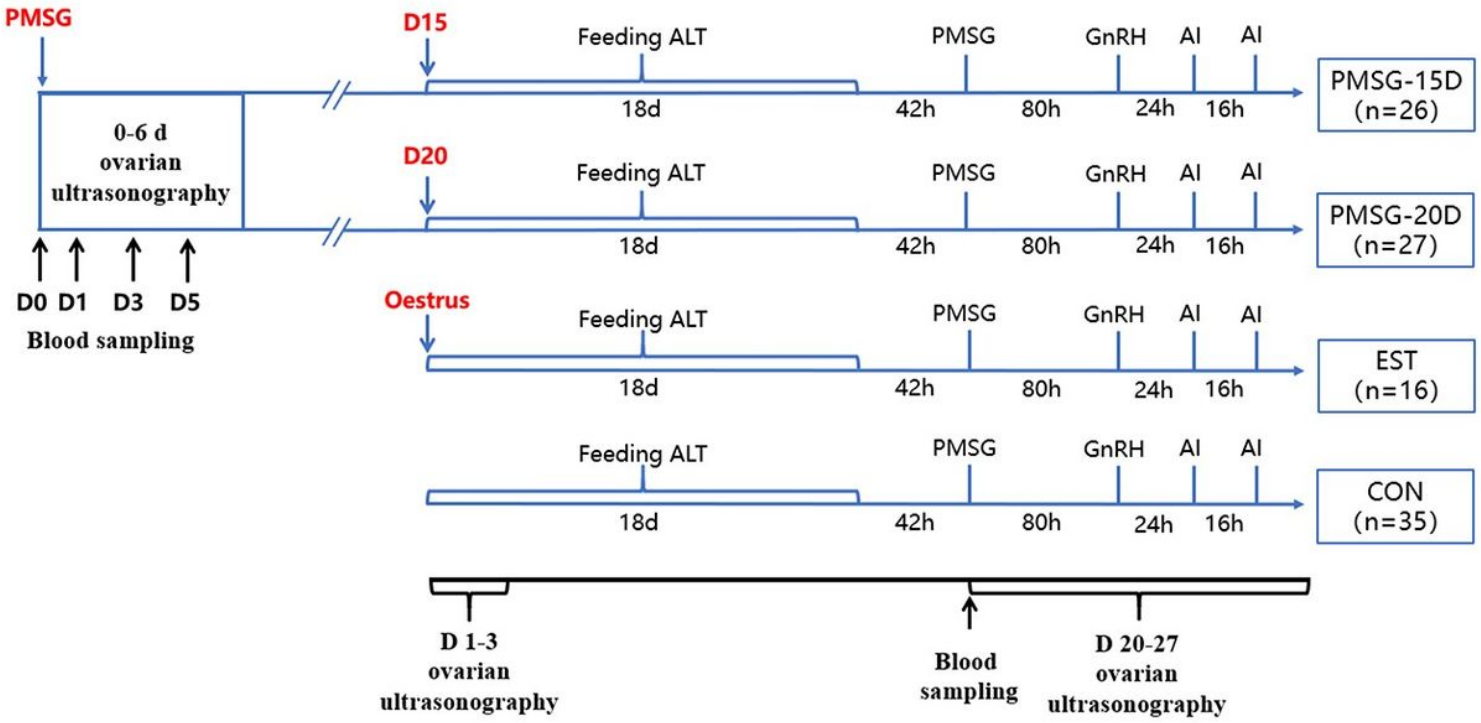

Figure 1

Schematic of experimental design

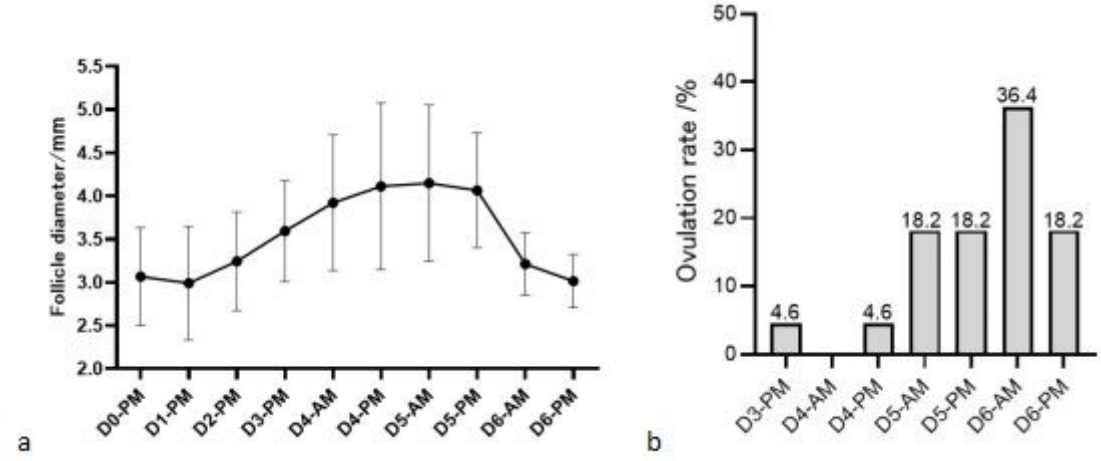

Figure 2

Follicular development in replacement gilts pretreated with PMSG. a, Follicular development trend after PMSG pretreatment. b, Distribution of ovulation time after PMSG pretreatment.

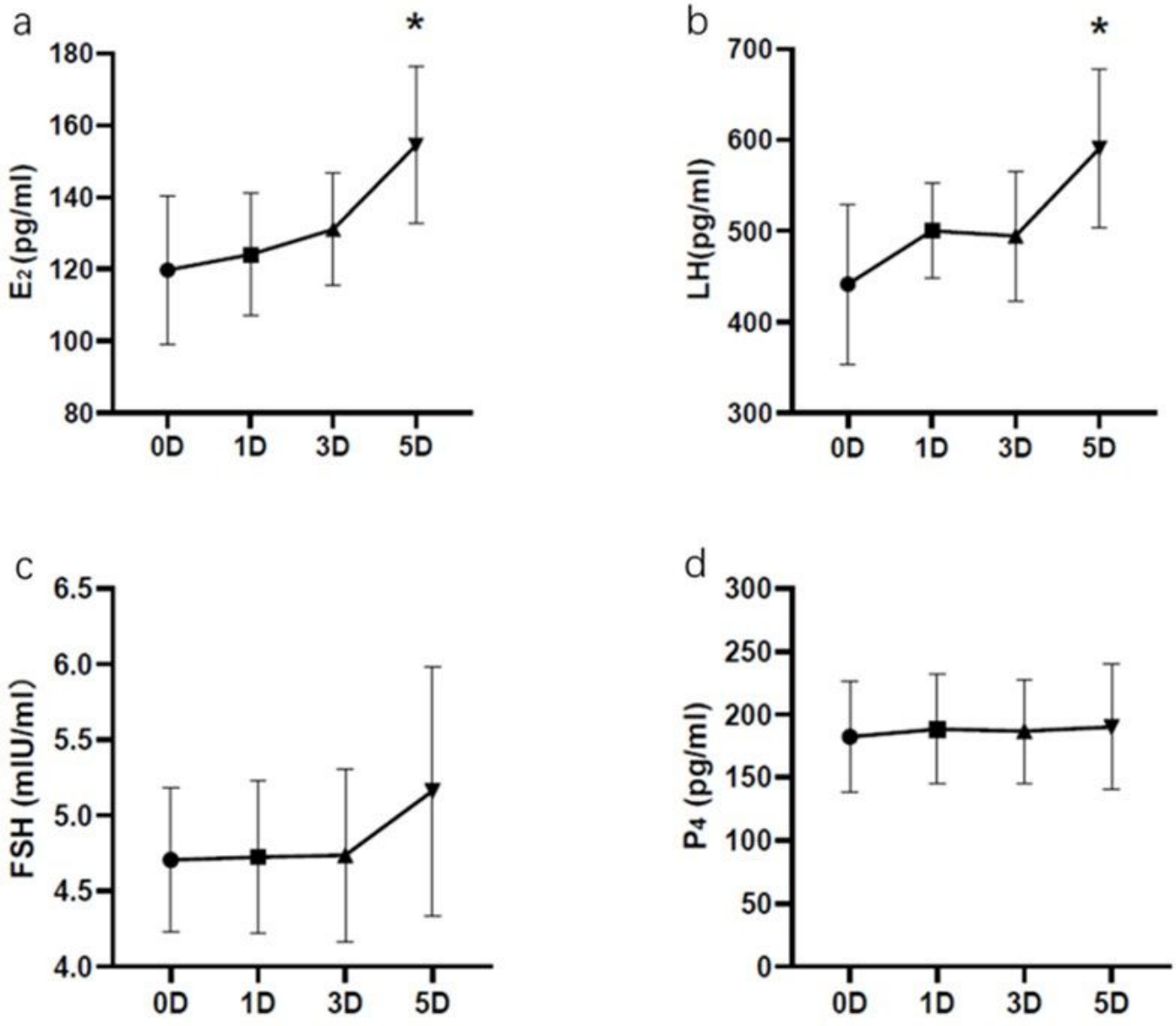

Figure 3

Hormone level changes on 0 day, 1 day, 3 day, 5 day after PMSG pretreatment. E2 (a), LH (b), FSH (c) and P4 (d)

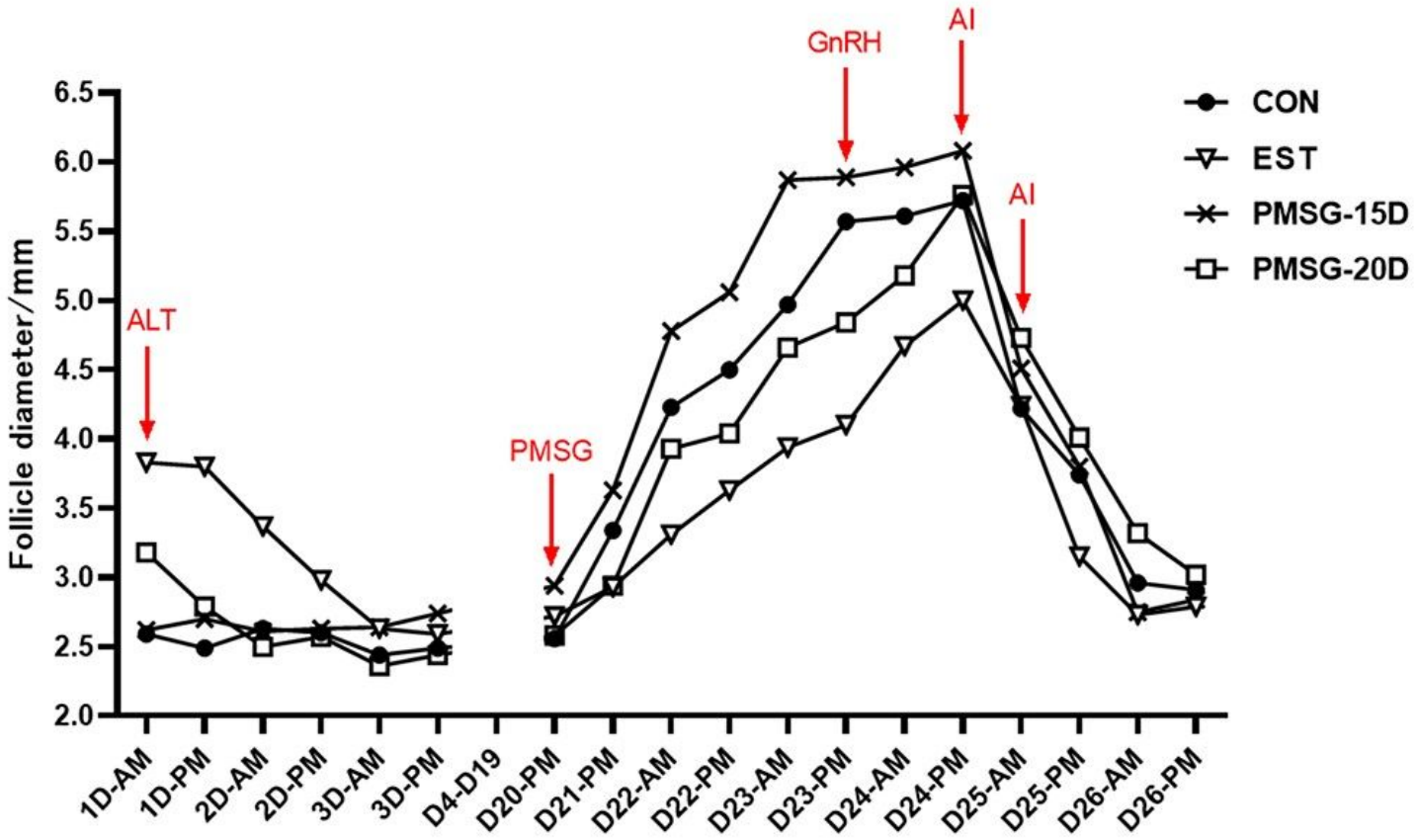

Figure 4

Follicular development trends of all groups after ALT feeding.

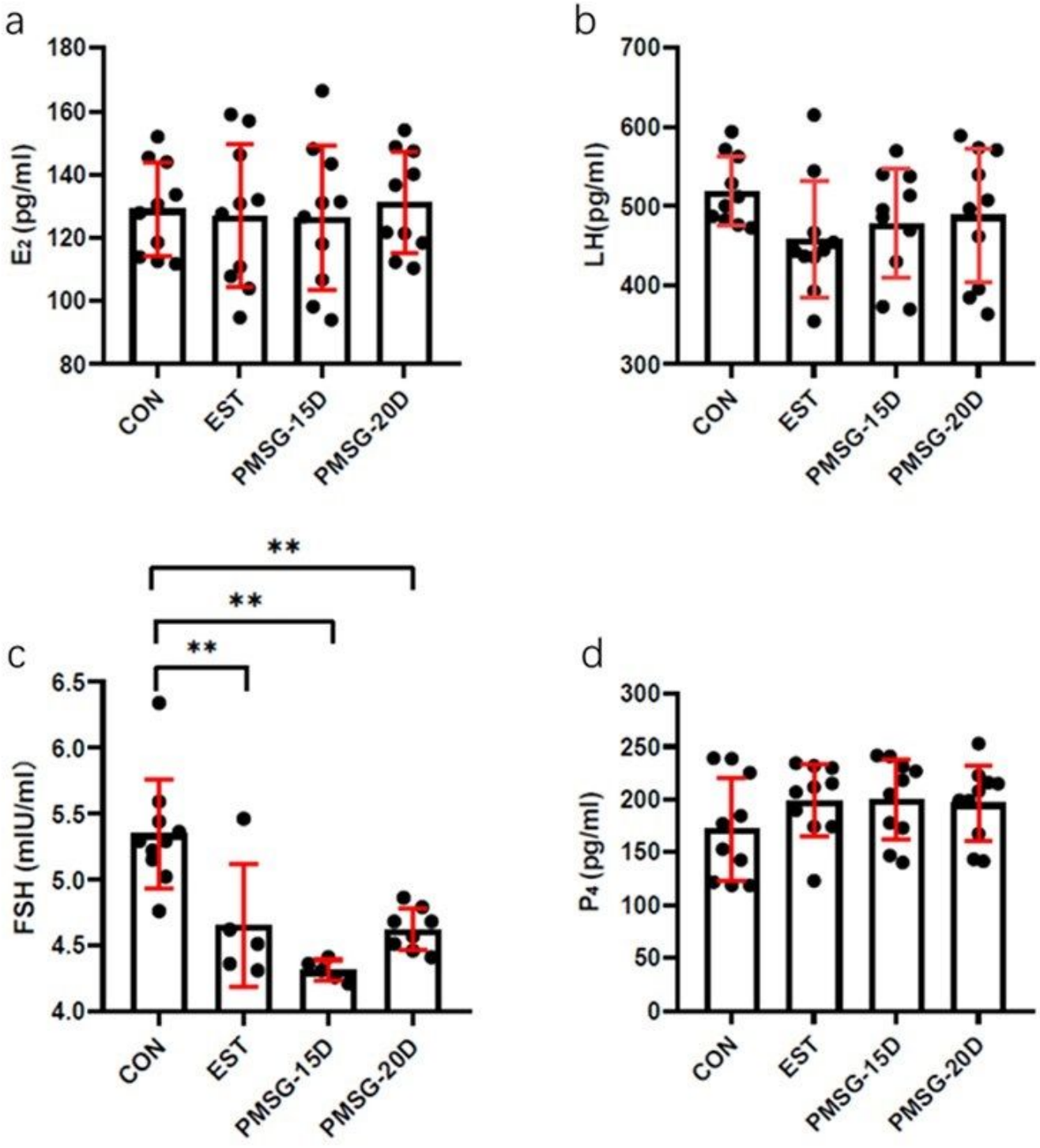

Figure 5

Hormone level changes across groups at 42 hours after ALT feeding stopped. E2 (a), LH (b), FSH (c) and P4 (d)

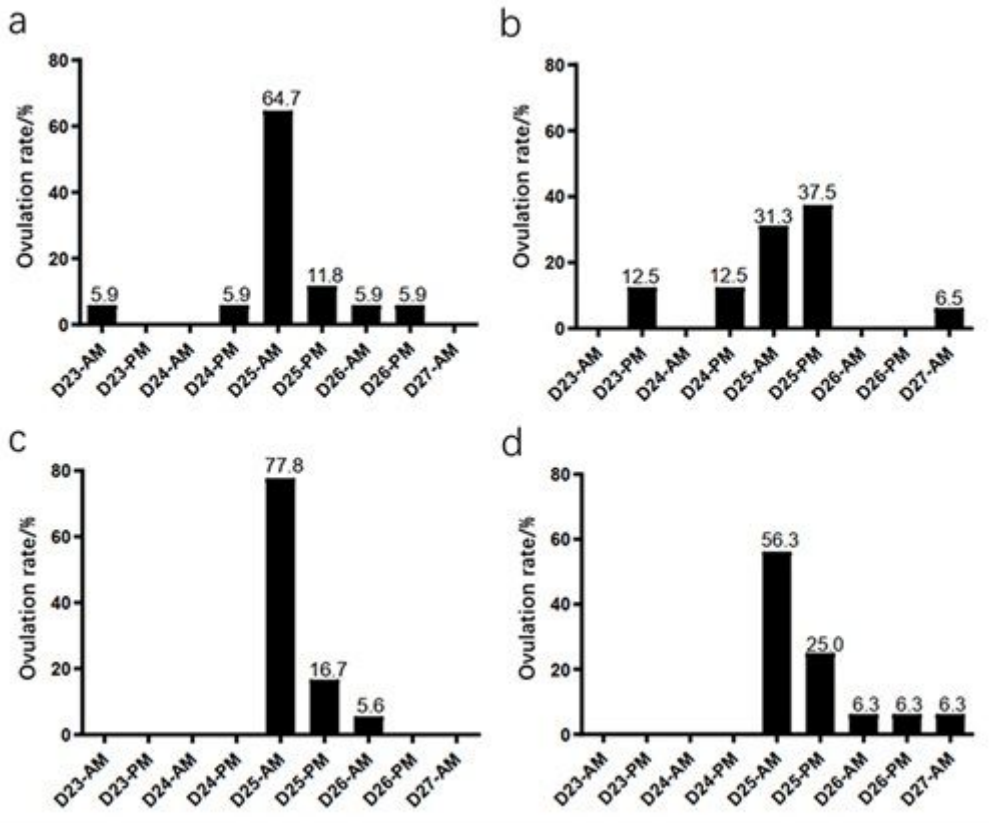

Figure 6

The proportion of ovulating gilts in different groups. CON (a), EST (b), PMSG-15D (c), and PMSG-20D (d).

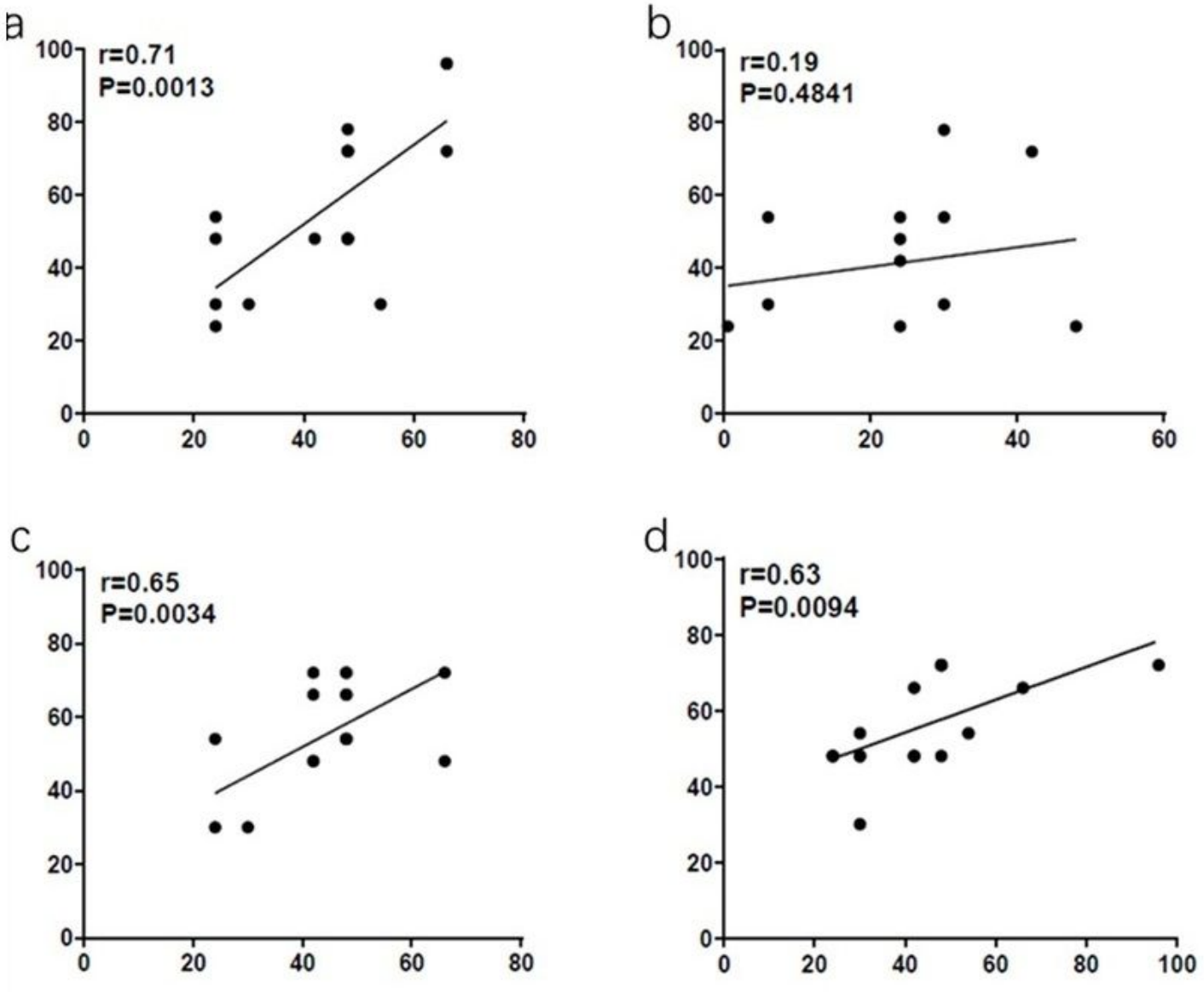

Figure 7

Correlation between estrus-ovulation interval and estrus duration in different groups. CON (a), EST (b), PMSG-15D (c), and PMSG-20D (d)