



# UNIVERSITÀ DI PARMA

## ARCHIVIO DELLA RICERCA

University of Parma Research Repository

Gilt reproductive performance in a tropical environment after oestrus synchronization and fixed-time artificial insemination

This is the peer reviewed version of the following article:

*Original*

Gilt reproductive performance in a tropical environment after oestrus synchronization and fixed-time artificial insemination / Tummaruk, P.; Sang-Gassanee, K.; Audban, C.; Pichitpantapong, S.; Panyathong, R.; Lin, H.; De Rensis, F.. - In: THERIOGENOLOGY. - ISSN 0093-691X. - 192:(2022), pp. 45-51. [10.1016/j.theriogenology.2022.08.026]

*Availability:*

This version is available at: 11381/2930231 since: 2022-11-23T08:33:25Z

*Publisher:*

*Published*

DOI:10.1016/j.theriogenology.2022.08.026

*Terms of use:*

Anyone can freely access the full text of works made available as "Open Access". Works made available

*Publisher copyright*

note finali coverpage

(Article begins on next page)

# Gilt reproductive performance in a tropical environment after oestrus synchronization and fixed-time artificial insemination

Padet Tummaruk<sup>a,f,\*</sup>, Kridtasak Sang-Gassanee<sup>b</sup>, Chairach Audban<sup>c</sup>, Somjit Pichitpantapong<sup>c</sup>, Raphee Panyathong<sup>c</sup>, Hongyao Lin<sup>d</sup>, Fabio De Rensis<sup>e</sup>

<sup>a</sup> Department of Obstetrics, Gynaecology and Reproduction, Faculty of Veterinary Science, Chulalongkorn University, Bangkok, 10330, Thailand

<sup>b</sup> Intervet (Thailand) Ltd., South Sathorn Rd., Yannawa, Sathorn, Bangkok, 10120, Thailand

<sup>c</sup> Charoen Pokphand Foods Public Company Limited, Bangkok, Thailand

<sup>d</sup> Merck Animal Health, S12-2206, 556 Morris Avenue, Summit, NJ, 07901, USA

<sup>e</sup> Department of Veterinary Medical Science, University of Parma, Parma, Italy

<sup>f</sup> Centre of Excellence in Swine Reproduction, Chulalongkorn University, Bangkok, 10330, Thailand

\*Corresponding author

*E-mail address:* Padet.t@chula.ac.th (P. Tummaruk)

## ABSTRACT

The present study was performed to evaluate the reproductive performances of gilts inseminated using fixed-time artificial insemination (FTAI) protocol without oestrus. A total of 408 Landrace × Yorkshire crossbred gilts were included in the experiment. The gilts at 8 months of age were randomly allocated into three groups: control AI (n = 192), treatment 1-TAI (n = 117) and treatment 2-FTAI (n = 99). Gilts in the control AI group were inseminated. Gilts in the treatment 1-TAI group were orally administered 20 mg per day of altrenogest for 18 days and then inseminated once a day at oestrus. Gilts in the treatment 2-FTAI group were synchronized like gilts in treatment 1-TAI group but then GnRH (10 µg of buserelin) was administered 120 h after the end of altrenogest treatment and fixed time artificial inseminated twice at 24 and 32 h after GnRH irrespective of the presence of oestrus or not. Conception rate of gilts in treatment 2-FTAI (87.9%) was similar to the treatment 1-TAI (94.9%) and control AI (83.3%) ( $P > 0.05$ ). Conception rate in treatment 1-TAI (94.9%) was higher compared to control AI group (83.3%,  $P = 0.040$ ). Farrowing rate of gilts in treatment 2-FTAI (83.8%) was similar to treatment 1-TAI (89.7%) and control AI (83.3%) ( $P > 0.05$ ). Farrowing rate of treatment 1-TAI (89.7%) was higher than control AI sows (76.0%,  $P = 0.033$ ). In treatment 2-FTAI, the conception and farrowing rate of the nine gilts that were inseminated even if they were not detected in oestrus (all during warm season) was 44.4% and 44.4%, respectively. Regular return to oestrus was similar between groups (9.4%, 0.9% and 4.1% for control AI, treatment 1-TAI and treatment 2-FTAI, respectively,  $P > 0.05$ ). The total number of piglets born per litter in treatment 1-TAI group was higher than control AI ( $13.1 \pm 0.2$  versus  $11.6 \pm 0.2$ , respectively,  $P < 0.001$ ) and treatment-2 FTAI groups ( $12.2 \pm 0.3$ ,  $P = 0.019$ ). The number of piglets born alive was higher in treatment 1-TAI ( $12.1 \pm 0.3$ ) compared to treatment 2-FTAI ( $11.3 \pm 0.2$ ) and control AI group ( $11.2 \pm 0.3$ ). The stillbirth and mummified foetus were not different between groups ( $P > 0.05$ ). The present study indicated that fixed-time AI in gilts can be performed by administration of altrenogest for 18 days, GnRH at 120 h after altrenogest withdrawal and then double fixed-time AI at 24 and 32 h after the administration of GnRH result in conception rate, farrowing rate and litter performances similar to gilts inseminated at oestrus with conventional AI.

**Keywords:** Altrenogest, Buserelin, FTAI, GnRH, Pig, Reproduction

50  
51  
52  
53  
54  
55  
56  
57  
58  
59  
60  
61  
62  
63  
64  
65  
66  
67  
68  
69  
70  
71  
72  
73  
74  
75  
76  
77  
78  
79  
80  
81  
82  
83  
84  
85  
86  
87  
88  
89  
90  
91  
92  
93  
94  
95  
96  
97  
98  
99

## 1. Introduction

Return-to-oestrus after artificial insemination is one of the most common infertility problems causing pregnancy failure in gilts raised under tropical environment. Under field conditions, the proportion of mated females returned-to-oestrus account for 16.3%, 9.9% and 6.7% in gilts, primiparous sows and sow parity numbers 2 – 14, respectively [1]. However, the proportion of female return-to-oestrus varies among herds from 5.0 to 10.5% [1]. Several research have tried to use gonadotropins, i.e. equine chorionic gonadotropin (eCG) and human Chorionic Gonadotropin (hCG) to improve fertility rate of gilt and sow [2 – 4]. In fact, administration of exogenous gonadotropin releasing hormone (GnRH) at the onset of standing oestrus can induce luteinizing hormone (LH) secretion from pituitary gland and result in ovulation at  $37.5 \pm 3.3$  h after the GnRH administration [5]. Similarly, administration of 750 IU of hCG at the onset of standing oestrus in sows can also induce ovulation  $40.2 \pm 1.7$  h later, while spontaneous ovulation occurs at about  $63.6 \pm 9.6$  h after standing oestrus [5]. Interestingly, without the use of exogenous GnRH or hCG, the timing of ovulation can be varied among individual sows from 33.0 to 99.0 h [5]. Thus, this variation can be one of the primary causes of return-to-oestrus problem. In gilts, Bracken et al. [6] demonstrated that the interval from the onset of oestrus to ovulation average 33.5 h and ovulation take place at 59.5% of the oestrus duration. Fertilization rate and number of embryos are maximized when the gilts are inseminated between 0 to 12 h before ovulation [6]. On the other hand, when the insemination was performed at >24 h before ovulation, the fertilization rate is below 40% [6]. These findings indicate that optimal doses of semen and optimal timing of insemination relative to ovulation in gilts are key factors for improving fertility rate in gilts.

With the aim to archive better synchronize the time of ovulation with insemination, recent studies in sow [2,4] and in gilts [7] together with previous study [3] show that it is possible to use eCG, GnRH and porcine luteinizing hormone (pLH) to synchronize ovulation in sow and gilts. When weaned sows are synchronized ovulation with eCG in combination with GnRH (buserelin) followed by fixed-time AI at 30 to 33 h after administration, farrowing rate is similar to control sows inseminated with conventional AI (82.2 versus 93.7% for treated and control sows, respectively) [2]. Moreover, sows treated with 10  $\mu$ g of buserelin at 86  $\pm$  3 h after weaning and are fixed time inseminated once at 30 to 33 h later results in a farrowing rate of 87.0% and 84.5% in the treated and control sows, respectively [8]. Moreover, litter size was also similar between treated and control sows (13.6 versus 13.7) [8].

In temperate and tropical area of the world gilts and sows usually present a reduction in fertility during the warm period of the year. Indeed, the farrowing rate of gilts raised in tropical countries and mated in the warm season (69.5%) is lower than gilts mated in rainy (73.1%) and cool seasons (76.8%) [1]. In sows exposed to 30 °C compared to sows exposed to 22 °C, LH pulse frequency at day 9 of lactation is reduced [9] and cortisol concentration of lactating sows was increased in the sows exposed to 30 °C compared to sows exposed to 22 °C [9]. These findings indicate that the female pigs exposed to elevated ambient temperature exhibited altered endocrine function and compromise farrowing rate. A recent study report that the threshold of the maximum temperature that led to a reduction in farrowing rate was 20, 21, and 24 to 25 °C for gilts, primiparous sows and multiparous sows, respectively [10]. An increase of daily maximum temperature during 2 to 3 weeks before service by 10 °C above these thresholds decreased farrowing rate by 3.0%, 4.3%, and 1.9% to 2.8%, for gilts, primiparous sows and multiparous sows, respectively [10]. Additionally, heat stress compromise also the production of the sows. Iida and Koketsu [11] demonstrated that the total number of piglets born per litter in gilts decreased by 0.05 piglets for each degree Celsius increase in preservice maximum temperature. Furthermore, in Thailand, seasonal influence on the litter size at birth of pigs are more pronounce in the gilts than sow litter [12]. All these data suggest that cooling management during preservice period in summer season has been recommended but even so, there is a compromission of the reproductive and productive performances of sows and gilts. These clinical observations indicate that reproductive management in gilts raised under tropical environment need to be improved.

100 In addition, sows weaned in warm season have a smaller follicle size both at weaning and at the onset of  
101 oestrus than those weaned in cold season [13]. Additionally, the proportion of sows with delayed ovulation  
102 is higher in warm than in cold season [13]. These findings indicates that tropical environments can  
103 compromise follicle development and ovulation time in gilts and sows. Thus, the control of follicle  
104 development and ovulation time may help to improve reproductive performance of gilts under tropical  
105 conditions. Therefore, the present study aims to determine the possibility to develop a protocol that allow  
106 artificial insemination at a predeterminate time without the need of oestrus detection (fixed-time artificial  
107 insemination - FTAI) in gilts. Because in sow and gilts under heat stress the oestrus behaviour can be  
108 compromised and thus the heat detection rate is low [14,15] this study also compared the effect on the  
109 reproductive performances of FTAI during the cold and warm periods of the year in a tropical country.  
110

## 111 2. Materials and methods

### 112 2.1 *Animals and experimental designs*

113  
114  
115 The experiment followed the guidelines documented in the Ethical Principles and Guidelines for the Use  
116 of Animals for Scientific Purposes, edited by the National Research Council of Thailand, and was approved  
117 by the Institutional Animal Care and Use Committee (IACUC) in accordance with the university regulations  
118 and policies governing the care and use of experimental animals, approval no. 1731011. A total of 408  
119 Landrace × Yorkshire crossbred gilts were included in the experiment. The gilts at 8 months of age were  
120 randomly allocated into three groups: control AI (n = 192), treatment 1-TAI (n = 117) and treatment 2-  
121 FTAI (n = 99). Gilts in the control AI group were inseminated at oestrus once a day. Gilts in treatment 1-  
122 TAI group was synchronized oestrus by orally administration of 20 mg per day of altrenogest (Regumate®,  
123 Merck Animal Health, NJ, USA) for 18 days to each individual gilts by using drenching gun at 0800 h daily  
124 and artificial insemination was effectuated at oestrus once a day. Gilts in the treatment 2-FTAI group were  
125 synchronized with altrenogest like gilts in group treatment 1-TAI and then followed by administration of  
126 GnRH (10 µg of buserelin, 2.5 ml buserelin 4.0 µg/ml, Receptal®, Merck Animal Health, NJ, USA) 120 h  
127 after altrenogest withdrawal. Thereafter, gilts were fixed time artificial inseminated (FTAI) twice, at 24 and  
128 32 h after the GnRH administration irrespective of the presence or absence of standing oestrus (Figure 1).  
129 Reproductive performance, i.e. conception rate at 28 days after AI, farrowing rate, total number of piglets  
130 born per litter (TB), number of piglets born alive per litter (BA), proportion of mummified foetuses (MF)  
131 and stillborn piglets (SB) were evaluated for each sow. In gilts that did not get pregnant, the interval from  
132 AI to return-to-oestrus was determined and the gilts that return to estrus at 18 to 24 days after AI was  
133 defined as regular return-to-oestrus (RR). Additionally, the influence of season was determined. Season of  
134 mating was classified into two groups: warm (May to September, n = 170) and cold (November-January; n  
135 = 238) seasons.  
136

### 137 2.2 *General management*

138  
139 The experiment was conducted in a commercial swine herd located in the Northern part of Thailand. The  
140 temperatures inside the barn during cold and warm seasons were  $21.7 \pm 4.8$  °C (range: 10.5 – 33.0) and  
141  $25.8 \pm 2.1$  °C (range: 20.5 – 35.0), respectively. The average humidity levels inside the barn in cold and  
142 warm seasons were  $88.8 \pm 9.1\%$  (range: 51.0 – 97.0) and  $87.8 \pm 6.6\%$  (range: 45.5 – 97.5), respectively.  
143 The gilts (8 months of age) were moved from the gilt pool to the mating house and kept in individual crates  
144 (0.6 × 2.0 m) on a concrete-slatted floor. The gilts were fed with 2.5–3.0 kg/day of a corn-soybean-based  
145 diet (15.0% crude protein, 0.85% digestible lysine and 3.0 Mcal/kg metabolisable energy). After  
146 insemination, the gilts were moved to the gestation house and kept in the individual crates until one week  
147 before farrowing. The gestating sows were fed with 1.5–3.5 kg of feed per day with a corn-soybean-based  
148 diet (16.0% crude protein, 1.0% digestible lysine and 2.8 Mcal/kg metabolisable energy). The gestation diet  
149 was gradually increased to 3.0–3.5 kg/day during 12–15 weeks of gestation, and the amount of feed was  
150 gradually declined during the last week of gestation. Gestating gilts were moved to the farrowing house

151 approximately one week before the expected date of parturition. The gilts were placed in individual crates  
152 ( $0.6 \times 2.2$  m) at the centre of the farrowing pens ( $2.0 \times 2.2$  m). The pens were fully slatted with a concrete  
153 base at the centre for sows and with steel slats at both sides of the farrowing crate for piglets. Lactating  
154 sows were fed with 5.0–6.0 kg/day of a corn-soybean-based diet containing 18.0% crude protein, 1.0%  
155 digestible lysine and 3.2 Mcal/kg metabolisable energy, 3–4 times a day. Water was provided ad libitum  
156 via water nipples. The backfat thickness of sows was measured via A-mode ultrasonography (Renco Lean-  
157 Meater<sup>®</sup>, Minneapolis, MN, USA) at farrowing, weaning and insemination. Measurements were taken at  
158 the level of the last rib at 6–8 cm from the midline, on both sides of the sows. The average between the left  
159 and the right sides was calculated [15].

160

### 161 *2.3 Oestrus detection, artificial insemination, and pregnancy detection*

162

163 Oestrus detection of the gilts was carried out twice a day at 0700h and 1600h starting from the day after  
164 they entry the gilt pool by experienced stockpersons using the back-pressure test in the presence of a mature  
165 boar. The gilts exhibiting clear standing responses in front of the boar, i.e. accepting the back-pressure test,  
166 arching their backs, erected ears and immobilised legs, were considered in oestrus. In all groups, a standard  
167 AI foam tip catheter was inserted into the vagina and fixed at the cervix to deposit a semen dose of  $3.0 \times$   
168  $10^9$  sperms in 100 ml volume. The pooled semen used for all gilts was obtained from two to three Duroc  
169 boars aged 1 to 3 years, collected by the gloved hand method. Only semen with a subjective motility above  
170 70% was used. Sperm concentration was evaluated using a spectrophotometer (Spermacue<sup>®</sup>, Minitübe  
171 GmbH, Tiefenbach, Germany). The ejaculates were diluted with a semen extender (M III<sup>®</sup>, Minitübe  
172 International AG, Germany) to obtain a final concentration of  $30 \times 10^6$  sperms per ml. Diluted semen was  
173 stored at 17 °C and were used within 24 h. To determine conception rate at 28 days after insemination,  
174 trans-abdominal B-mode ultrasonography (HS-2000, Honda Electronics Co. Ltd., Oiwa-cho Toyohashi,  
175 Aichi, Japan) was used.

176

### 177 *2.4 Statistical analysis*

178

179 The statistical analyses were carried out by using SAS version 9.4 (SAS Inst. Inc., Cary, NC, USA.).  
180 Descriptive statistics on reproductive data of gilts in control AI, treatment 1-TAI and treatment 2-FTAI  
181 groups were calculated by using the MEANS procedure of SAS. Frequency analysis on categorical traits,  
182 i.e. regular return-to-oestrus rate, conception rate and farrowing rate was carried out using the FREQ  
183 procedure of SAS. Categorical data (i.e. regular return-to-oestrus rate, conception rate and farrowing rate)  
184 was compared by logistic regression using GLIMMIX procedure of SAS. The factors included in the  
185 statistical models included group (control AI, treatment 1-TAI and treatment 2-FTAI), season (cold or  
186 warm) and two-ways interaction. Least square means were obtained from each class of the variables and  
187 compared by using the least significant difference test. Continuous data (i.e. TB, BA, SB and MF) were  
188 analysed by using the general linear model (GLM) procedure of SAS. The statistical models included fixed  
189 effects of group (control AI, treatment 1-TAI and treatment 2-FTAI), season (cold or warm) and two-ways  
190 interaction. Least square means were obtained from each class of the variables and compared by using the  
191 least significant difference test. In addition, the average backfat thickness was compared between gilts that  
192 exhibited standing heat within 32 h after buserelin administration and gilts that did not exhibit standing heat  
193 by using Student' *t* test. Moreover, the regular return-to-oestrus rate, conception rate and farrowing rate of  
194 gilts that exhibited standing oestrus within 32 h after GnRH administration and gilts that did not exhibit  
195 standing oestrus were analysed by using Chi-square test. In the treatment 2-FTAI group, litter traits (i.e.,  
196 TB, BA, SB and MF) were compared between gilts exhibited standing oestrus at insemination and gilts that  
197 did not exhibit standing oestrus at insemination by using one way ANOVA. Categorical traits (i.e. regular  
198 return-to-oestrus rate, conception rate and farrowing rate) were also compared between gilts exhibited  
199 standing oestrus at insemination and those that did not exhibited standing oestrus at insemination by using  
200 Chi-square test. For all analyses, a *P* value of less than 0.05 indicated statistical significance.

201

202  
203  
204  
205  
206  
207  
208  
209  
210  
211  
212  
213  
214  
215  
216  
217  
218  
219  
220  
221  
222  
223  
224  
225  
226  
227  
228  
229  
230  
231  
232  
233  
234  
235  
236  
237  
238  
239  
240  
241  
242  
243  
244  
245  
246  
247  
248  
249  
250  
251  
252

### 3. Results

#### 3.1 Descriptive data

On average, gestation length of all the gilts included in the experiment were  $115.1 \pm 1.7$  days (range: 109 – 122). There were not differences between groups ( $P > 0.05$ ). The backfat thickness of gilts at insemination and at 109 days of gestation were  $16.2 \pm 3.5$  and  $19.6 \pm 3.5$  mm, respectively and were not differences between groups ( $P > 0.05$ ). The backfat thickness was not different ( $P = 0.308$ ) between gilts that did not exhibit standing heat ( $14.5 \pm 2.4$ ) and gilts that exhibited standing heat ( $15.5 \pm 2.8$  mm). In control AI and treatment 1-TAI groups, the number of inseminations during standing oestrus (means  $\pm$  SD) were  $2.7 \pm 0.5$  and  $2.7 \pm 0.5$  times, respectively. The proportion of gilts inseminated for three times during standing oestrus were 70.8% and 71.8% in the control AI and treatment 1-TAI groups, respectively (Table 1). The temperature inside the barn during warm season was  $4.2$  °C higher than cold season ( $25.8 \pm 2.1$  and  $21.7 \pm 4.7$  °C, respectively,  $P < 0.001$ ). Relative humidity during warm season was 1.0% lower than cold season ( $87.8 \pm 6.6\%$  and  $88.8 \pm 9.1\%$ , respectively,  $P = 0.006$ ). The proportion of days when the temperature inside the barn raised above  $25.0$  °C in warm season was higher than cold season (52.4% and 25.6%, respectively,  $P < 0.001$ ).

#### 3.2 Fertility data

Reproductive performances of gilts in control AI, treatment 1-TAI and treatment 2-FTAI are presented in Table 1. There were not differences in conception rate between treatment-2 FTAI (87.9%) and the other two groups (Table 1). Conception rate of gilts in treatment-1 TAI (94.9%) was higher than control AI group (83.3%,  $P = 0.040$ ). Similarly, farrowing rate was not different between treatment 2-FTAI (83.8%) and the other two groups (Table 1). Farrowing rate of gilts in treatment 1-TAI (89.7%) was higher than control AI (76.0%,  $P = 0.033$ ). In treatment 2-FTAI, the conception and farrowing rate of the gilts that exhibited standing oestrus ( $n = 90$ ) was 92.2% and 87.8%, respectively (Figure 2), for the nine gilts that were inseminated even if were not detected in oestrus (all occurring during warm season), conception rate was 44.4% and farrowing rate was 44.4% (Figure 2). Regular return-to-oestrus was similar between groups (9.4% and 4.1% in control AI, treatment 1-TAI and treatment 2-FTAI, respectively,  $P > 0.05$ ) (Table 1). Conception rate, farrowing rate and regular return-to-oestrus rate of gilts in control AI, treatment 1-TAI and treatment 2-FTAI in cold and warm seasons are presented in Table 2. In cold season, the farrowing rate in either treatment 1-TAI or treatment 2-FTAI was higher than control AI groups (Table 2). In warm season, farrowing rate in treatment 1-TAI were higher than control AI and treatment 2-FTAI (Table 2).

#### 3.3 Productive performances (litter size at birth)

On average, the TB, BA, SB and MF were  $12.1 \pm 2.5$ ,  $11.1 \pm 2.4$ , 4.3% and 2.3%, respectively. The TB, BA, SB and MF of gilts in control AI, treatment 1-TAI and treatment 2-FTAI are presented in Table 1. The TB in treatment 1-TAI group was higher than control AI ( $13.1 \pm 0.2$  versus  $11.6 \pm 0.2$ , respectively,  $P < 0.001$ ) and treatment 2-FTAI groups ( $12.2 \pm 0.3$ ,  $P = 0.019$ ). Likewise, the BA was higher in treatment 1-TAI ( $12.1 \pm 0.3$ ) compared with treatment 2-FTAI ( $11.3 \pm 0.2$ ,  $P = 0.017$ ) and control AI group ( $11.2 \pm 0.3$ ,  $P < 0.001$ ) (Table 2). The SB and MF were not different between groups (Table 2). The TB, BA, SB and MF of gilts in control AI, treatment 1-TAI and treatment 2-FTAI in cold and warm seasons are presented in Table 2. In cold season, the TB and BA in treatment 2-FTAI group was similar to the other two groups (Table 2). On the other hand, in warm season, TB and BA in treatment 1-TAI and treatment 2-FTAI were higher than control AI (Table 2). The SB and MF were similar between groups (Table 2). The TB, BA, SB and MF of the gilts in treatment 2-FTAI group that were inseminated when the animals exhibited standing oestrus ( $n = 83$  gilts) was  $12.2 \pm 0.3$ ,  $11.2 \pm 0.3$ , 4.7% and 1.8%, respectively.

253 In the treatment-2 FTAI, four gilts that were fixed-time artificially inseminated even if they did not show  
254 oestrus conceived and farrowed. The TB, BA, SB and MF of these four gilts were  $10.5 \pm 1.3$ ,  $10.5 \pm 1.3$ ,  
255 0% and 0%, respectively and was not different from the sow fixed-time artificially inseminated in the  
256 presence of oestrus ( $P > 0.05$ ).

257

#### 258 4. Discussion

259

260 One aim of this study is to evaluate the conception rate in gilts submitted to a fixed-time artificial  
261 insemination protocol, thus avoiding the need of heat detection. Our result indicates that fixed-time artificial  
262 insemination can be applied also in gilts because conception and farrowing rate of treatment 2-FTAI gilts  
263 was similar to the control AI and treatment 1-TAI in which gilts were inseminated only in the presence of  
264 oestrus symptoms. In a contemporary to our study, a recent study in Spain [7] has been observed that gilts  
265 synchronized with altrenogest and inseminated with a single fixed-time AI at 30-33 h after GnRH  
266 (buserelin) administration using post-cervical insemination with 60 ml of  $1500 \times 10^6$  sperm per dose,  
267 resulted in a farrowing rate of 91.1% and a total number of piglets born of 18.5 piglets per litter and there  
268 were no difference with the control group inseminated using conventional AI. In China, replacement gilts  
269 pre-treated with eCG 15 days before subjected to fixed-time artificial insemination programme have a  
270 farrowing rate of 90% and a total number of piglets born per litter of 11.6 [16] that were not different from  
271 gilts inseminated after oestrus detection. Thus, we confirm and improve these observations that fixed-time  
272 artificial insemination can be applied in gilts. Moreover, we have shown that the gilts that did not show  
273 standing oestrus but are fixed-time artificial inseminated (4/9) can become pregnant and that the number of  
274 TB, BA, MF and SB is similar to gilts inseminated at oestrus.

275

276 In the present study, all the gilts that did not exhibit standing oestrus within 32 h after GnRH administration  
277 occurred in the warm season. Similarly, a previous study in sows have demonstrated that the percentage of  
278 sows that ovulated between 44–56 h after GnRH administration is higher in the cold season compared to  
279 warm season [17]. An experiment carried out in a temperate-region farm in Spain indicate that sows having  
280 small ovarian follicles ( $< 5$  mm) at the time of GnRH (buserelin) treatment have a poorer response to the  
281 treatment, and this is more evident in sows weaned during summer–autumn period [18]. These data  
282 indicated that season of the year significantly influence the response of gilts to GnRH treatment. In a  
283 previous study, the incidence of sows that do not show oestrus symptoms within 8 days after GnRH  
284 (buserelin) administration was 12.8% and none of the anestrus sows ovulated [18]. This evidence was  
285 frequently observed in primiparous and in second parity sows [18]. Different from the study of Lopez et al.  
286 [18], in our study in treatment 2-FTAI group 44.4% of gilts that were not in oestrus get pregnant after FTAI.  
287 This difference could be related to the fact that we considered gilts and not sow, and that the animals were  
288 pre-synchronized with altrenogest. We have no ultrasound data that can help us to determine the ovarian  
289 activity at the moment of treatment, but at least for the gilts that get pregnant, the ovary and follicle has to  
290 be responsive to GnRH administration.

291

292 Finally, in the present study, the utilization of altrenogest for 18 days resulted in a better reproductive  
293 performances of gilts (+1.3 piglets and 13.7% improvement in farrowing rate compared control gilts). This  
294 confirms previous study in which synchronization of gilts by altrenogest has a positive effect on  
295 reproductive performances [3]. Tummaruk et al. (2009) has reported that the most common culling reason  
296 in replacement gilts in tropical environment is due to anoestrus and up to 52.2% of the gilts culled due to  
297 anoestrus had inactive ovaries and that poor oestrus symptom is the major reason causing poor reproductive  
298 performance in replacement gilts. Thus, when the oestrus was controlled by altrenogest administration, the  
299 reproductive performance of gilts was significantly improved.

300

#### 301 5. Conclusion

302

303 The present study indicated that fixed-time AI in gilts can be performed by administration of altrenogest  
304 for 18 days, buserelin at 120 h after withdrawal altrenogest and then double fixed-time AI at 24 and 32 h  
305 after the administration of GnRH. This protocol resulted in conception rate, farrowing rate and productive  
306 performances (TB, BA, MF and SB) similar to gilts inseminated at oestrus after a conventional AI or after  
307 synchronization with altrenogest. We also observed that some gilts, that were not detected oestrus but were  
308 fixed-time artificially inseminated, become pregnant and farrowed and this effect was evident during the  
309 warm season thus suggesting that fixed-time artificial insemination may reduce the negative impact on  
310 fertility of heat stress.

311

## 312 **Acknowledgements**

313

314 This research was funded by the Thailand Science Research and Innovation Fund, Chulalongkorn  
315 University (CU\_FRB65\_food (19)183\_31\_02) and MSD Animal Health. P. Tummaruk received a grant  
316 from the Thailand Science Research and Innovation (RTA6280013). Chulalongkorn University supported  
317 the One Health Research Cluster. The authors are grateful to Charoen Pokphand Foods Public Co. Ltd. and  
318 their staff for helping with animal management.

319

## 320 **References**

321

- 322 [1] Tummaruk P, Tantasuparuk W, Techakumphu M, Kunavongkrit A. Influence of repeat-service and  
323 weaning-to-first-service interval on farrowing proportion of gilts and sows. *Prev Vet Med*  
324 2010;96:194–200. <https://doi.org/10.1016/j.prevetmed.2010.06.003>
- 325 [2] Baroncello E, Bernardi ML, Kummer AD, Wentz I, Bortolozzo FP. Fixed-time post-cervical artificial  
326 insemination in weaned sows following buserelin use combined with/without eCG. *Reprod Dom Anim*  
327 2017;52:76–82. <https://doi.org/10.1111/rda.12805>
- 328 [3] De Rensis F, Kirkwood RN. Control of oestrus and ovulation: Fertility to timed insemination of gilts  
329 and sows. *Theriogenology* 2016;86:1460–6. <http://dx.doi.org/10.1016/j.theriogenology.2016.04.089>
- 330 [4] Pearodwong P, Tretipskul C, Panyathong R, Sang-Gassanee K, Collell M, Muns R, Tummaruk P.  
331 Reproductive performance of weaned sows after single fixed-time artificial insemination under a  
332 tropical climate: Influences of season and insemination technique. *Theriogenology* 2020;142:54–61.  
333 <https://doi.org/10.1016/j.theriogenology.2019.09.032>
- 334 [5] Wongkawewit K, Prommachart P, Raksasub R, Buranaamnuy K, Techakumphu M, De Rensis F,  
335 Tummaruk P. Effect of the administration of GnRH or hCG on time of ovulation and the onset of  
336 oestrus-to-ovulation interval in sows in Thailand. *Trop Anim Health Prod* 2012;44:467–70.  
337 <https://doi.org/10.1007/s11250-011-9920-3>
- 338 [6] Bracken CJ, Safranski TJ, Cantley TC, Lucy MC, Lamberson WR. Effect of time of ovulation and  
339 sperm concentration on fertilization rate in gilts. *Theriogenology* 2003;60:669–76.  
340 [https://doi.org/10.1016/S0093-691X\(03\)00085-2](https://doi.org/10.1016/S0093-691X(03)00085-2)
- 341 [7] Suárez-Usbeck A, Mitjana O, Tejedor MT, Bonastre C, Sistac J, Ubierno A, Falceto MV. Single fixed-  
342 time post-cervical insemination in gilts with buserelin. *Animals* 2021;11:1567.  
343 <https://doi.org/10.3390/ani11061567>
- 344 [8] Driancourt MA, Cox P, Rubion S, Harnois-Milon G, Kemp B, Soede NM. Induction of an LH surge  
345 and ovulation by buserelin (as Receptal) allows breeding of weaned sows with a single fixed-time  
346 insemination. *Theriogenology* 2013;80:391–9. <http://dx.doi.org/10.1016/j.theriogenology.2013.05.002>
- 347 [9] Barb CR, Estienne MJ, Kraeling RR, Marple DN, Rampacek GB, Rahe CH, Sartin JL. Endocrine  
348 changes in sows exposed to elevated ambient temperature during lactation. *Domest Anim Endocrinol*  
349 1991;8:117–27. [https://doi.org/10.1016/0739-7240\(91\)90046-M](https://doi.org/10.1016/0739-7240(91)90046-M)
- 350 [10] Iida R, Pineiro C, Koketsu Y. Timing and temperature thresholds of heat stress effects on fertility  
351 performance of different parity sows in Spanish herds. *J Anim Sci* 2021;99:skab173.  
352 <https://doi.org/10.1093/jas/skab173>



- 353 [11] Iida R, Koketsu Y. Interactions between pre- or postservice climatic factors, parity and weaning-to-  
354 first-mating interval for total number of pigs born of female pigs serviced during hot and humid or cold  
355 seasons. *J Anim Sci* 2014;92:4180–8. <https://doi.org/10.2527/jas.2014-7636>
- 356 [12] Tummaruk P, Tantasuparuk W, Techakumphu M, Kunavongkrit A. Seasonal influence on the litter  
357 size at birth of pig are more pronounced in the gilt than sow litter. *J Agri Sci* 2010;148:421–32.  
358 <https://doi.org/10.1017/S0021859610000110>
- 359 [13] Lopes TP, Sanchez-Osorio J, Bolarin A, Martinez EA, Roca J. Relevance of ovarian follicular  
360 development to the seasonal impairment of fertility in weaned sows. *Vet J* 2014;199:382–6.  
361 <https://doi.org/10.1016/j.tvjl.2013.11.026>
- 362 [14] De Rensis F, Ziecik AJ, Kirkwood RN. Seasonal infertility in gilts and sows: Aetiology, clinical  
363 implications and treatments. *Theriogenology* 2017;96:111–7.  
364 <https://doi.org/10.1016/j.theriogenology.2017.04.004>
- 365 [15] Pearodwong P, Tretipskul C, Panyathong R, Tummaruk P. Factors influencing pre-ovulatory follicle  
366 diameter and weaning-to-ovulation interval in spontaneously ovulating sows in tropical environment.  
367 *Reprod Dom Anim* 2020;55:1756–63. <https://doi.org/10.1111/rda.13836>
- 368 [16] Zhao Q, Tao C, Pan J, Wei Q, Zhu Z, Wang L, Liu M, Huang J, Yu F, Chen X, Zhang L, Li J. Equine  
369 chorionic gonadotropin pretreatment 15 days before fixed-time artificial insemination improves the  
370 reproductive performance of replacement gilts. *Animal* 2021;15:100406.  
371 <https://doi.org/10.1016/j.animal.2021.100406>
- 372 [17] Pearodwong P, Tretipskul C, Soede NM, Tummaruk P. Factors affecting estrus and ovulation time in  
373 weaned sows with induced ovulation by GnRH administration in different seasons. *J Vet Med Sci*  
374 2019;81:1567–74. <https://doi.org/10.1292/jvms.18-0429>
- 375 [18] Lopez TP, Padilla L, Bolarin A, Rodriquez-Martinez H, Roca J. Weaned sows with small ovarian  
376 follicles respond poorly to the GnRH agonist buserelin. *Animals* 2020;10:1979.  
377 <http://dx.doi.org/10.3390/ani10111979>
- 378 [19] Tummaruk P, Kedsangsakonwut S, Kunavongkrit A. Relationships among specific reason for culling,  
379 reproductive data and gross-morphology of the genital tracts in gilts culled due to reproductive failure  
380 in Thailand. *Theriogenology* 2009;71:369–75. <https://doi.org/10.1016/j.theriogenology.2008.08.003>
- 381  
382

383 **Table 1**  
 384 Reproductive performance of gilts that had conventional artificial insemination (control AI) compared with  
 385 gilts synchronized using altrenogest and inseminate at oestrus (treatment 1-TAI) or gilts (treatment 2-FTAI)  
 386 synchronized using altrenogest, GnRH and inseminated at fixed-time AI 24 and 32 h after GnRH (least-  
 387 square means  $\pm$  SEM).  
 388

Variables	Control AI	Treatment 1-TAI	Treatment 2-FTAI
<i>Number of gilts inseminated</i>	192	117	99
Number of inseminations	2.7 $\pm$ 0.5	2.7 $\pm$ 0.5	2.0 $\pm$ 0.0
Regular return-to-oestrus (%)	9.4 <sup>a</sup>	0.9 <sup>a</sup>	4.1 <sup>a</sup>
Conception rate at 28 days (%)	83.3 <sup>a</sup>	94.9 <sup>b</sup>	87.9 <sup>ab</sup>
Farrowing rate (%)	76.0 <sup>a</sup>	89.7 <sup>b</sup>	83.8 <sup>ab</sup>
<i>Number of gilts that farrow</i>	146	105	83
Total number of piglets born/ litter	11.8 $\pm$ 0.4 <sup>a</sup>	13.1 $\pm$ 0.2 <sup>b</sup>	12.2 $\pm$ 0.3 <sup>a</sup>
Number of piglets born alive/ litter	11.2 $\pm$ 0.3 <sup>a</sup>	12.1 $\pm$ 0.2 <sup>b</sup>	11.3 $\pm$ 0.2 <sup>a</sup>
Stillbirth (%)	2.7 $\pm$ 1.4 <sup>a</sup>	4.7 $\pm$ 0.9 <sup>a</sup>	4.7 $\pm$ 1.0 <sup>a</sup>
Mummified foetuses (%)	1.6 $\pm$ 0.9 <sup>a</sup>	2.2 $\pm$ 0.6 <sup>a</sup>	1.7 $\pm$ 0.7 <sup>a</sup>

389 <sup>a,b</sup> Different superscripts within row differ significantly ( $P < 0.05$ )  
 390  
 391

392 **Table 2**  
 393 Reproductive performance of gilts that had conventional artificial insemination (control AI) compared with  
 394 gilts synchronized using altrenogest and inseminate at oestrus (treatment 1-TAI) or gilts (treatment 2-FTAI)  
 395 synchronized using altrenogest, GnRH and inseminated at fixed-time AI 24 and 32 h after GnRH (least-  
 396 square means  $\pm$  SEM) considering the season of treatment (cold, Nov – Jan or warm, Apr – Jul).  
 397

Variables	Control AI	Treatment 1-TAI	Treatment 2-FTAI
<b>Cold season</b>			
<i>Number of gilts inseminated</i>	112	72	54
Regular return-to-oestrus (%)	7.1 <sup>a</sup>	0.0 <sup>a</sup>	0.0 <sup>a</sup>
Conception rate at 28 days (%)	83.0 <sup>a</sup>	94.4 <sup>b</sup>	90.7 <sup>ab</sup>
Farrowing rate (%)	73.2 <sup>a</sup>	87.5 <sup>b</sup>	87.0 <sup>b</sup>
<i>Number of gilts that farrow</i>			
Total number of piglets born/ litter	11.7 $\pm$ 0.3 <sup>ab</sup>	12.3 $\pm$ 0.3 <sup>b</sup>	11.3 $\pm$ 0.3 <sup>a</sup>
Number of piglets born alive/ litter	10.7 $\pm$ 0.2 <sup>a</sup>	11.2 $\pm$ 0.3 <sup>a</sup>	10.5 $\pm$ 0.3 <sup>a</sup>
Stillbirth (%)	2.4 $\pm$ 1.0 <sup>a</sup>	6.0 $\pm$ 1.1 <sup>b</sup>	3.2 $\pm$ 1.4 <sup>a</sup>
Mummified foetuses (%)	3.4 $\pm$ 0.7 <sup>a</sup>	1.7 $\pm$ 0.8 <sup>a</sup>	1.6 $\pm$ 0.9 <sup>a</sup>
<b>Warm season</b>			
<i>Number of gilts inseminated</i>	80	45	45
Regular return-to-oestrus (%)	12.5 <sup>a</sup>	2.2 <sup>a</sup>	8.9 <sup>a</sup>
Conception rate at 28 days (%)	83.8 <sup>a</sup>	95.6 <sup>a</sup>	84.4 <sup>a</sup>
Farrowing rate (%)	80.0 <sup>a</sup>	93.3 <sup>b</sup>	80.0 <sup>ab</sup>
<i>Number of gilts that farrow</i>			
Total number of piglets born/ litter	11.4 $\pm$ 0.3 <sup>a</sup>	13.9 $\pm$ 0.4 <sup>b</sup>	13.2 $\pm$ 0.4 <sup>b</sup>
Number of piglets born alive/ litter	10.3 $\pm$ 0.3 <sup>a</sup>	13.0 $\pm$ 0.3 <sup>b</sup>	12.1 $\pm$ 0.4 <sup>b</sup>
Stillbirth (%)	5.5 $\pm$ 1.2 <sup>a</sup>	3.5 $\pm$ 1.4 <sup>a</sup>	6.1 $\pm$ 1.6 <sup>a</sup>
Mummified foetuses (%)	2.2 $\pm$ 0.8 <sup>a</sup>	2.6 $\pm$ 1.0 <sup>a</sup>	1.8 $\pm$ 1.0 <sup>a</sup>

398 <sup>a,b</sup> Different superscripts within row differ significantly ( $P < 0.05$ )  
 399  
 400  
 401

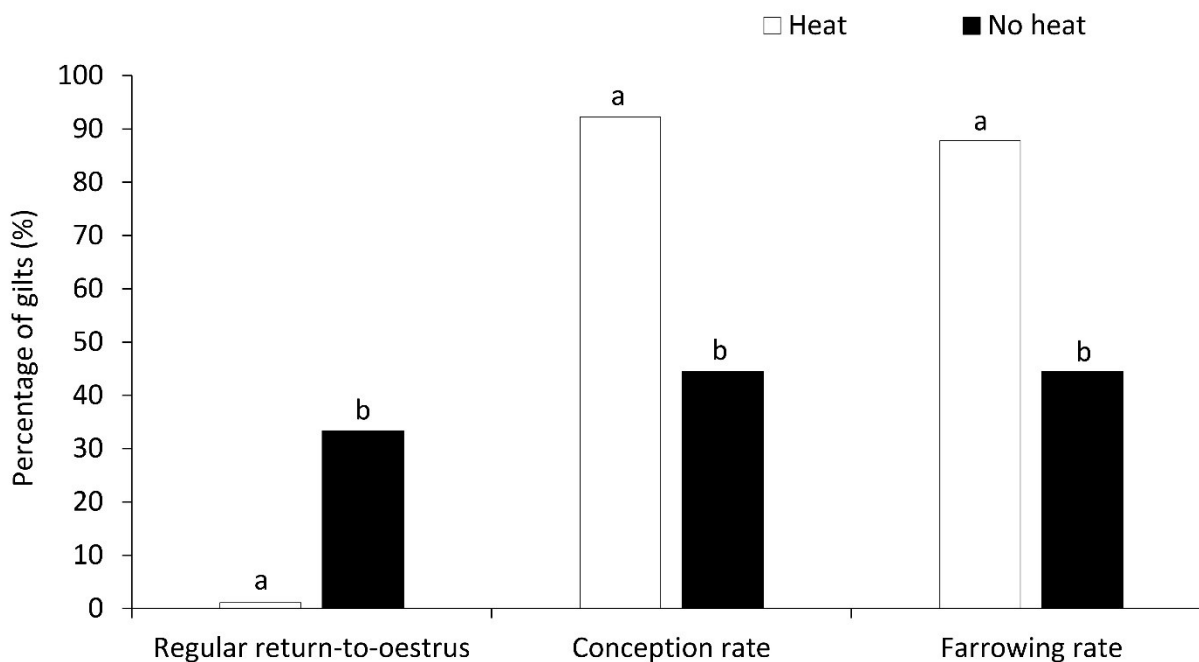
402 Fig 1. Experimental protocol. Control-AI group: sows inseminated 2 – 3 times during standing oestrus at  
 403 0, 12 and 24 h after the onset of oestrus. Group TAI: orally administered 20 mg per day of altrenogest for  
 404 18 days and then inseminated at oestrus by conventional AI. Group FTAI: sow synchronized like gilts in  
 405 treatment 1-TAI group but then GnRH (10  $\mu$ g of buserelin) was administered 120 h after the end of  
 406 altrenogest treatment and fixed time artificial inseminated twice at 24 and 32 h after GnRH irrespective of  
 407 the presence of oestrus or not.  
 408  
 409



410  
411  
412  
413  
414  
415  
416  
417  
418  
419  
420  
421  
422  
423  
424  
425  
426  
427  
428

429  
430  
431  
432

**Figure 1** Protocol for fixed-time artificial insemination in gilt



433  
434  
435  
436  
437  
438

**Figure 2** Conception and farrowing rate and regular return-to-oestrus in treatment 2-FTAI group (oestrus synchronization by altrenogest and GnRH and fixed time artificial insemination 24 and 32 h after GnRH) in presence (heat) or in absence (no heat) of oestrus, <sup>a,b</sup> Different superscripts within group differ significantly ( $P < 0.05$ )