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

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## 1 Gilt reproductive performance in a tropical environment after oestrus

### 2 synchronization and fixed-time artificial insemination

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- 4
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#### 18 19 ABSTRACT

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21 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 22 23 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 24 25 control AI group were inseminated. Gilts in the treatment 1-TAI group were orally administered 20 mg per 26 day of altrenogest for 18 days and then inseminated once a day at oestrus. Gilts in the treatment 2-FTAI 27 group were synchronized like gilts in treatment 1-TAI group but then GnRH (10 µg of buserelin) was 28 administered 120 h after the end of altrenogest treatment and fixed time artificial inseminated twice at 24 29 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 30 31 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%) 32 33 (P > 0.05). Farrowing rate of treatment 1-TAI (89.7%) was higher than control AI sows (76.0%, P = 0.033). 34 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 35 to oestrus was similar between groups (9.4%, 0.9% and 4.1% for control AI, treatment 1-TAI and treatment 36 37 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 38 39  $(12.2 \pm 0.3, P = 0.019)$ . 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 40 foetus were not different between groups (P>0.05). The present study indicated that fixed-time AI in gilts 41 can be performed by administration of altrenogest for 18 days, GnRH at 120 h after altrenogest withdrawal 42 43 and then double fixed-time AI at 24 and 32 h after the administration of GnRH result in conception rate, 44 farrowing rate and litter performances similar to gilts inseminated at oestrus with conventional AI.

- 44 45
- 46 Keywords: Altrenogest, Buserelin, FTAI, GnRH, Pig, Reproduction
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#### 50

#### 51 1. Introduction

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53 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 54 females returned-to-oestrus account for 16.3%, 9.9% and 6.7% in gilts, primiparous sows and sow parity 55 56 numbers 2 - 14, respectively [1]. However, the proportion of female return-to-oestrus varies among herds 57 from 5.0 to 10.5% [1]. Several research have tried to use gonadotropins, i.e. equine chorionic gonadotropin 58 (eCG) and human Chorionic Gonadotropin (hCG) to improve fertility rate of gilt and sow [2-4]. In fact, 59 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 60 61 the GnRH administration [5]. Similarly, administration of 750 IU of hCG at the onset of standing oestrus 62 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 63 ovulation can be varied among individual sows from 33.0 to 99.0 h [5]. Thus, this variation can be one of 64 65 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 66 67 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 68 69 ovulation, the fertilization rate is below 40% [6]. These findings indicate that optimal doses of semen and 70 optimal timing of insemination relative to ovulation in gilts are key factors for improving fertility rate in 71 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

 $\pm$  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].

- 81 In temperate and tropical area of the world gilts and sows usually present a reduction in fertility during the 82 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 83 exposed to 30 °C compared to sows exposed to 22 °C, LH pulse frequency at day 9 of lactation is reduced 84 85 [9] and cortisol concentration of lactating sows was increased in the sows exposed to 30 °C compared to 86 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 87 the threshold of the maximum temperature that led to a reduction in farrowing rate was 20, 21, and 24 to 88 89 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 90 91 by 3.0%, 4.3%, and 1.9% to 2.8%, for gilts, primiparous sows and multiparous sows, respectively [10]. 92 Additionally, heat stress compromise also the production of the sows. Iida and Koketsu [11] demonstrated 93 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 94 at birth of pigs are more pronounce in the gilts than sow litter [12]. All these data suggest that cooling 95 96 management during preservice period in summer season has been recommended but even so, there is a
- 97 compromission of the reproductive and productive performances of sows and gilts. These clinical
- 98 observations indicate that reproductive management in gilts raised under tropical environment need to be
- 99 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 development and ovulation time may help to improve reproductive performance of gilts under tropical 104 conditions. Therefore, the present study aims to determine the possibility to develop a protocol that allow 105 106 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 compromised and thus the heat detection rate is low [14,15] this study also compared the effect on the 108 reproductive performances of FTAI during the cold and warm periods of the year in a tropical country. 109

#### 111 2. Materials and methods

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113 2.1 Animals and experimental designs

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

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#### 137 2.2 General management

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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  $25.8 \pm 2.1$  °C (range: 20.5 – 35.0), respectively. The average humidity levels inside the barn in cold and 141 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. 142 The gilts (8 months of age) were moved from the gilt pool to the mating house and kept in individual crates 143  $(0.6 \times 2.0 \text{ m})$  on a concrete-slatted floor. The gilts were fed with 2.5–3.0 kg/day of a corn-soybean-based 144 diet (15.0% crude protein, 0.85% digestible lysine and 3.0 Mcal/kg metabolisable energy). After 145 146 insemination, the gilts were moved to the gestation house and kept in the individual crates until one week before farrowing. The gestating sows were fed with 1.5–3.5 kg of feed per day with a corn-soybean-based 147 diet (16.0% crude protein, 1.0% digestible lysine and 2.8 Mcal/kg metabolisable energy). The gestation diet 148 149 was gradually increased to 3.0-3.5 kg/day during 12-15 weeks of gestation, and the amount of feed was gradually declined during the last week of gestation. Gestating gilts were moved to the farrowing house 150

151 approximately one week before the expected date of parturition. The gilts were placed in individual crates  $(0.6 \times 2.2 \text{ m})$  at the centre of the farrowing pens  $(2.0 \times 2.2 \text{ m})$ . The pens were fully slatted with a concrete 152 153 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% digestible lysine and 3.2 Mcal/kg metabolisable energy, 3-4 times a day. Water was provided ad libitum 155 via water nipples. The backfat thickness of sows was measured via A-mode ultrasonography (Renco Lean-156 157 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].

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

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

177 2.4 Statistical analysis

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

## 203204 3. Results

- 204
- 206 *3.1 Descriptive data*
- 207

208 On average, gestation length of all the gilts included in the experiment were  $115.1 \pm 1.7$  days (range: 109 209 -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 210 between groups (P > 0.05). The backfat thickness was not different (P = 0.308) between gilts that did not 211 exhibit standing heat  $(14.5 \pm 2.4)$  and gilts that exhibited standing heat  $(15.5 \pm 2.8 \text{ mm})$ . In control AI and 212 213 treatment 1-TAI groups, the number of inseminations during standing oestrus (means  $\pm$  SD) were 2.7  $\pm$  0.5 214 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 215 temperature inside the barn during warm season was 4.2 °C higher than cold season (25.8  $\pm$  2.1 and 21.7  $\pm$ 216 4.7 °C, respectively, P < 0.001). Relative humidity during warm season was 1.0% lower than cold season 217  $(87.8 \pm 6.6\% \text{ and } 88.8 \pm 9.1\%, \text{ respectively}, P = 0.006)$ . The proportion of days when the temperature inside 218 219 the barn raised above 25.0 °C in warm season was higher than cold season (52.4% and 25.6%, respectively, 220 *P*<0.001).

- 221
- 222 *3.2 Fertility data*
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224 Reproductive performances of gilts in control AI, treatment 1-TAI and treatment 2-FTAI are presented in 225 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 226 227 (83.3%, P = 0.040). Similarly, farrowing rate was not different between treatment 2-FTAI (83.8%) and the 228 other two groups (Table 1). Farrowing rate of gilts in treatment 1-TAI (89.7%) was higher than control AI 229 (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 230 inseminated even if were not detected in oestrus (all occurring during warm season), conception rate was 231 232 44.4% and farrowing rate was 44.4% (Figure 2). Regular return-to-oestrus was similar between groups 233 (9.4% and 4.1% in control AI, treatment 1-TAI and treatment 2-FTAI, respectively, P > 0.05) (Table 1). 234 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 235 in either treatment 1-TAI or treatment 2-FTAI was higher than control AI groups (Table 2). In warm season, 236 237 farrowing rate in treatment 1-TAI were higher than control AI and treatment 2-FTAI (Table 2).

- 238
- 239 *3.3 Productive performances (litter size at birth)*
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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.

In the treatment-2 FTAI, four gilts that were fixed-time artificially inseminated even if they did not show oestrus conceived and farrowed. The TB, BA, SB and MF of these four gilts were  $10.5 \pm 1.3$ ,  $10.5 \pm 1.3$ , 0% and 0%, respectively and was not different from the sow fixed-time artificially inseminated in the 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 insemination can be applied also in gilts because conception and farrowing rate of treatment 2-FTAI gilts 262 was similar to the control AI and treatment 1-TAI in which gilts were inseminated only in the presence of 263 264 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 (buserelin) administration using post-cervical insemination with 60 ml of  $1500 \times 10^6$  sperm per dose, 266 resulted in a farrowing rate of 91.1% and a total number of piglets born of 18.5 piglets per litter and there 267 were no difference with the control group inseminated using conventional AI. In China, replacement gilts 268 pre-treated with eCG 15 days before subjected to fixed-time artificial insemination programme have a 269 270 farrowing rate of 90% and a total number of piglets born per litter of 11.6 [16] that were not different from gilts inseminated after oestrus detection. Thus, we confirm and improve these observations that fixed-time 271 272 artificial insemination can be applied in gilts. Moreover, we have shown that the gilts that did not show standing oestrus but are fixed-time artificial inseminated (4/9) can become pregnant and that the number of 273 274 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 occurred in the warm season. Similarly, a previous study in sows have demonstrated that the percentage of 277 278 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 treatment, and this is more evident in sows weaned during summer-autumn period [18]. These data 281 indicated that season of the year significantly influence the response of gilts to GnRH treatment. In a 282 283 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. This difference could be related to the fact that we considered gilts and not sow, and that the animals were 287 288 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.

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Finally, in the present study, the utilization of altrenogest for 18 days resulted in a better reproductive 292 performances of gilts (+1.3 piglets and 13.7% improvement in farrowing rate compared control gilts). This 293 294 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 in replacement gilts in tropical environment is due to anoestrus and up to 52.2% of the gilts culled due to 296 anoestrus had inactive ovaries and that poor oestrus symptom is the major reason causing poor reproductive 297 performance in replacement gilts. Thus, when the oestrus was controlled by altrenogest administration, the 298 299 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

for 18 days, buserelin at 120 h after withdrawal altrenogest and then double fixed-time AI at 24 and 32 h

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

- warm season thus suggesting that fixed-time artificial insemination may reduce the negative impact on
- 310 fertility of heat stress.
- 311

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#### 383 Table 1

Reproductive performance of gilts that had conventional artificial insemination (control AI) compared with gilts synchronized using altrenogest and inseminate at oestrus (treatment 1-TAI) or gilts (treatment 2-FTAI)

synchronized using altrenogest, GnRH and inseminated at fixed-time AI 24 and 32 h after GnRH (least-square means ± SEM).

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Variables	Control AI	Treatment 1-TAI	Treatment 2-FTAI
Number of gilts inseminated	192	117	99
Number of inseminations	$2.7\pm0.5$	$2.7\pm0.5$	$2.0\pm0.0$
Regular return-to-oestrus (%)	9.4ª	0.9ª	4.1 <sup>a</sup>
Conception rate at 28 days (%)	83.3ª	94.9 <sup>b</sup>	87.9 <sup>ab</sup>
Farrowing rate (%)	76.0ª	89.7 <sup>b</sup>	83.8 <sup>ab</sup>
Number of gilts that farrow	146	105	83
Total number of piglets born/ litter	$11.8\pm0.4^{\rm a}$	$13.1\pm0.2^{\text{b}}$	$12.2\pm0.3^{\rm a}$
Number of piglets born alive/ litter	$11.2\pm0.3^{\rm a}$	$12.1\pm0.2^{\text{b}}$	$11.3\pm0.2^{\rm a}$
Stillbirth (%)	$2.7\pm1.4^{\rm a}$	$4.7\pm0.9^{\rm a}$	$4.7 \pm 1.0^{\mathrm{a}}$
Mummified foetuses (%)	$1.6\pm0.9^{\mathrm{a}}$	$2.2\pm0.6^{\rm a}$	$1.7\pm0.7^{\mathrm{a}}$

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

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#### 392 Table 2

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

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Variables	Control AI	Treatment 1-TAI	Treatment 2-FTAI
Cold season			
Number of gilts inseminated	112	72	54
Regular return-to-oestrus (%)	7.1ª	0.0 <sup>a</sup>	$0.0^{a}$
Conception rate at 28 days (%)	83.0ª	94.4 <sup>b</sup>	90.7 <sup>ab</sup>
Farrowing rate (%)	73.2ª	87.5 <sup>b</sup>	87.0 <sup>b</sup>
Number of gilts that farrow	82	63	47
Total number of piglets born/ litter	$11.7\pm0.3^{\text{ab}}$	$12.3\pm0.3^{\text{b}}$	$11.3\pm0.3^{\rm a}$
Number of piglets born alive/ litter	$10.7\pm0.2^{\rm a}$	$11.2\pm0.3^{\rm a}$	$10.5\pm0.3^{\rm a}$
Stillbirth (%)	$2.4\pm1.0^{\rm a}$	$6.0 \pm 1.1^{b}$	$3.2\pm1.4^{\rm a}$
Mummified foetuses (%)	$3.4\pm0.7^{\rm a}$	$1.7\pm0.8^{\mathrm{a}}$	$1.6\pm0.9^{\rm a}$
Warm season			
Number of gilts inseminated	80	45	45
Regular return-to-oestrus (%)	12.5ª	2.2ª	8.9ª
Conception rate at 28 days (%)	83.8ª	95.6ª	84.4 <sup>a</sup>
Farrowing rate (%)	80.0ª	93.3 <sup>b</sup>	$80.0^{ab}$
Number of gilts that farrow	64	42	36
Total number of piglets born/ litter	$11.4\pm0.3^{\rm a}$	$13.9\pm0.4^{\text{b}}$	$13.2\pm0.4^{\rm b}$
Number of piglets born alive/ litter	$10.3\pm0.3^{\rm a}$	$13.0\pm0.3^{\text{b}}$	$12.1\pm0.4^{\rm b}$
Stillbirth (%)	$5.5\pm1.2^{\rm a}$	$3.5\pm1.4^{\rm a}$	$6.1 \pm 1.6^{\mathrm{a}}$
Mummified foetuses (%)	$2.2\pm0.8^{\rm a}$	$2.6 \pm 1.0^{\mathrm{a}}$	$1.8 \pm 1.0^{\mathrm{a}}$

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

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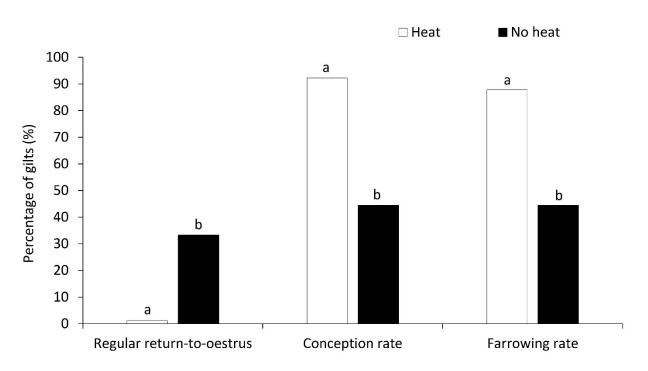
Fig 1. Experimental protocol. Control-AI group: sows inseminated 2-3 times during standing oestrus at 0, 12 and 24 h after the onset of oestrus. Group TAI: orally administered 20 mg per day of altrenogest for 18 days and then inseminated at oestrus by conventional AI. Group FTAI: sow synchronized like 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.

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Figure 1 Protocol for fixed-time artificial inseminated in gilt



434 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)