EFFECT OF THE TIMING OF OVUGEL® ADMINISTRATION ON REPRODUCTIVE PERFORMANCE IN GILTS SYNCHRONIZED FOR ESTRUS

BY

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DISSERTATION

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ABSTRACT

The objective of this thesis was to evaluate the effects of the time of OvuGel® administration on the timing of estrus and ovulation and fertility results in gilts synchronized for estrus by administering Matrix® for 14 d. Insemination must occur near the time of ovulation to achieve high fertility results, however, there is no practical method available to determine when ovulation occurs, and, therefore, sows and gilts are commonly inseminated 2 to 3 times over the course of the estrus period. Two exogenous hormones (Matrix® and OvuGel®) have been developed to aid in the process of estrus detection and insemination. Matrix® (Merck Animal Health, Summit, NJ) is an orally active progestogen that is used to synchronize estrus in gilts and OvuGel® (JBS United, Sheridan, IN) is a product that has been shown to synchronize ovulation in sows, allowing for a single fixed-time insemination to be used. A review of the literature relating to the timing of estrus and ovulation and the effects of Matrix® and OvuGel® administration on fertility results in gilts and sows was carried out. Overall, the findings of the review suggest that the timing of estrus and ovulation are highly variable in gilts and sows and could be dependent on a number of factors. In addition, the findings of the review suggest that gilts administered Matrix® had similar fertility results compared to gilts that were not administered Matrix®, and sows administered OvuGel® had similar fertility results compared to sows that were not administered OvuGel®. There has been no published research; however, evaluating the effect of OvuGel® administration in gilts on timing of estrus, ovulation, or fertility results. In a typical commercial situation, groups of gilts are in estrus and ovulating at different times, however, Matrix® administration would facilitate the use of OvuGel® and fixed-time insemination in a group of gilts. Little is known, however, of the effect of Matrix® administration in combination with OvuGel® administration and fixed-time insemination on

either the timing of estrus and ovulation or fertility results in gilts. A study was carried out, therefore, to determine the effect of the time of OvuGel® administration on timing of estrus and ovulation and fertility results in gilts synchronized for estrus using Matrix®. This study showed that the timing of OvuGel® administration had a significant effect on both the timing of estrus and ovulation and on subsequent pregnancy and farrowing rates and litter size. Further research is needed to establish the optimum time for administration of OvuGel® in gilts that have been synchronized for estrus.

Keywords: Gilt, OvuGel®, Ovulation, Estrus, Fertility

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CHAPTER 1: LITERATURE REVIEW

Introduction

Critical components of fertility in the pig are the period of estrus and, also, the process of insemination. It is commonly understood that insemination must occur near the time of ovulation for high fertility results to ensue; however, ovulation cannot be detected visually. Due to this inability to determine the time of ovulation, it is common practice to artificially inseminate a female 2 to 3 times over the course of an estrus period and deposit between 3 and 5 billion sperm cells into the reproductive tract for each insemination. Two exogenous hormones (OvuGel® and Matrix®) that are commercially available in the United States have been developed to aid in the process of estrus detection and insemination. OvuGel® (JBS United, Sheridan, IN) is a synthetic GnRH agonist that is approved for use in sows and is used to synchronize ovulation in an entire group of females, with the goal of allowing for one fixed-time insemination for each female. To date, however, research has focused on the effects of this product when used to synchronize ovulation in sows. Little is known, therefore, of the use of this product to effectively synchronize ovulation and ensure high fertility results in gilts. Altrenogest (Matrix®) has been widely adopted in the swine industry to synchronize estrus in a group of gilts, and may serve as a tool to facilitate the utilization of OvuGel® in gilts. This review will focus on discussing 1) the optimal timing of insemination, 2) characterization of the timing and variation of estrus and ovulation, and 3) the effect of Matrix® and OvuGel® on the synchronization of estrus and ovulation, and on fertility results in gilts and sows.

Time of insemination relative to ovulation

At the time of insemination, sperm cells will begin to migrate toward the caudal region of the isthmus in the oviduct, where they can be stored for up to 40 hours without a reduction in

fertilizing capability (Hunter, 1981; Pollard et al., 1991; Raycoudhurry and Suarez, 1991). However, only a small number of sperm cells actually reach the isthmus compared to the number that is deposited in the reproductive tract at insemination, and the remaining sperm cells are lost through either backflow or phagocytosis (Pursel et al., 1978).

Accessory sperm cells are defined as the sperm cells that become trapped in the zona pellucida of the oocyte after penetration of the first sperm cell (DeJarnette et al., 1992). The number of accessory sperm cells present at the time of fertilization has been used to estimate the total population of sperm cells that pass through the barriers of the reproductive tract to the location of storage and/or fertilization (Weitze et al., 1988; Saacke et al., 1994) and, therefore, represent the number of sperm cells present at the time of fertilization (after ovulation). To evaluate the impact of the interval from insemination to ovulation on the accessory sperm cell number, Steverink et al. (1997) measured the numbers of accessory sperm cells present 120 hours after ovulation in multiparous sows. They reported that there was a relationship between the insemination to ovulation interval and accessory sperm cell number; with a larger median number (19.7) of accessory sperm cells present when sows were inseminated 12 to 24 hours prior to ovulation compared with the number of accessory sperm cells present (8.3) when the insemination to ovulation interval was 24 to 36 hours. Soede et al. (1995) reached a similar conclusion, reporting that the highest accessory sperm cell number was reached when insemination occurred in the time interval from 8 hours prior to ovulation to 8 hours after ovulation.

It is well established that the time of insemination relative to time of ovulation is crucial in attaining the highest fertilization rates in both gilts and sows. In general, results of a number of studies suggest that the highest fertilization rates can be obtained when both gilts and sows are

inseminated in the time interval from 24 hours prior to ovulation and 4 hours after ovulation (Waberski et al., 1994; Nissen et al., 1997; Soede et al., 1995; Steverink et al., 1997; Bracken et al., 2003). However, 2 studies were conducted which reported a numerical reduction in pregnancy rates when insemination occurred outside of an even shorter time interval prior to insemination. Bortolozzo et al. (2005) reported an 11.1% reduction in pregnancy rate in pre-pubertal gilts inseminated greater than 16 hours prior to ovulation. These differences described by Bortolozzo et al. (2005), however, may be attributed to the use of pre-pubertal gilts in that study as opposed to post-pubertal gilts. Eliasson (1989) reported that proestrus in gilts was longer at the first estrus period than the second or third and that the first estrus period was significantly longer than the second, highlighting a potentially higher degree of variation or irregularity in the first estrus period. Similar to the results reported by Bortolozzo et al. (2005), Waberski et al. (1994) reported that pregnancy rates decreased by 20% in post-pubertal gilts inseminated more than 12 hours before ovulation. However, in this study (Waberski et al., 1994), only a small number of gilts (n = 5) were inseminated between 16 and 12 hours prior to ovulation, and there were no gilts that were inseminated greater than 16 hours before ovulation which raises questions about the validity of the approach used in this study.

In summary, there are some differing conclusions when recommending the optimum timing of insemination for any breeding female. The underlying conclusion, however, is that although inseminations may be administered throughout the course of an entire estrus, insemination must occur in the time interval from 24 hours prior to ovulation to 4 hours after ovulation for the highest fertilization rates to ensue. Although estrus can be detected visually through the use of boar stimuli and back pressure testing, the time of ovulation cannot be detected visually. All of the aforementioned studies utilized ultrasound to determine the time of

ovulation, but ultrasound is not practical in a commercial breeding herd. Having the ability to reliably predict the time of ovulation, and thus be able to time insemination around this event would greatly aid in reaching optimal fertility results in sows and gilts.

Variability in the timing of estrus and ovulation

It is typical in commercial practice for producers to visually check an entire group of females for behavioral estrus 1 to 2 times daily, as previously described. The highest conception rates and litter sizes can be obtained when insemination occurs in the interval from 24 hours prior to 4 hours after ovulation, however, as also previously described, ovulation cannot be detected visually. If a producer were able to predict the time of ovulation, then 1 insemination near the time of ovulation could be performed rather than having to check a female multiple times for estrus and perform multiple inseminations. Therefore, having the ability to accurately predict the time of ovulation would greatly improve the efficiency of insemination and be a useful tool to producers.

Soede and Kemp et al. (1997) reported that, on average, ovulation takes place 70% of the way through estrus and as a result suggested that the current best predictor for time of ovulation is the duration of estrus. In support of this claim, 3 studies have reported equations to predict the time of ovulation based on the duration of estrus as follows:

Ovulation time (multiparous sows) = 8.6 + 0.5 × duration of estrus (Steverink et al., 1997; R^2 = 59%).

Ovulation time (gilts) = Duration of estrus × 0.409 + 22.7 (Almeida et al., 2000; R^2 = 57%)

Ovulation time (gilts) = 0.5 × duration of estrus + 7.3 (Bortolozzo et al., 2005; R^2 = 40%).

Two important factors to consider however, when interpreting these relationships are that there were no validation studies completed utilizing any of these equations to test the accuracy of prediction of time of ovulation. Additionally, across the 3 studies, the equations based on duration of estrus accounted for only 40 to 59% of the variation in the time of estrus, indicating that other variables were responsible for a significant amount of variation.

A number of studies which have reported on duration of estrus, time from onset of estrus to time of ovulation, and time of ovulation within estrus in gilts and sows are summarized in Table 1.1. Of the 9 studies reporting duration of estrus, 4 of these reported duration of estrus in gilts; and 6 reported duration of estrus in multiparous sows (1 study reported results for both gilts and sows). Steverink et al. (1999) is the only study that evaluated both gilts and sows, and they reported a shorter duration of estrus for gilts. Because none of the other studies evaluated both gilts and sows, it is impossible to compare results between them; however, based on the studies reported, there appears to be no clear difference in estrus duration between gilts and sows. In general, the mean for the duration of estrus across the 4 studies evaluating gilts was 48.8 hours, although 3 of the 4 studies reported means greater than 50 hours while Steverink et al. (1999) reported a lower duration of estrus (41.2 hours). One reason for the shorter estrus duration in the latter study may be that it was conducted on a very large number of farms, therefore, potentially introducing more variation into the results (length of time observations were recorded, herd size, etc.) compared to the 3 other studies. In addition, Steverink et al. (1999) only carried out estrus detection twice daily and this would be less accurate in detecting the start and end of estrus compared to detecting estrus 3 to 4 times daily (as in the other studies). Finally, for the 6 studies

reported in Table 1.1 that used multiparous sows, the mean duration of estrus was 55.7 hours. Similar to the studies reporting duration of estrus for gilts, there was some degree of variation in the results obtained across studies (~10 hours difference in means across the studies).

Perhaps of greater importance than the differences between the means obtained across the studies in Table 1.1 is the degree of individual pig variation within study. In general, within a study, the reported range of difference between individual pigs for the duration of estrus was 19 to 38 hours less than or greater than the reported mean. Therefore, knowing the mean duration of estrus for an entire population of animals would not be useful when trying to determine the time of ovulation for each animal in the population, because in order to obtain the highest fertility results it is necessary to be able to predict time of ovulation (thus the proper time of insemination) for each animal. In addition, as mentioned earlier, the frequency of estrus detection and the time between each estrus detection is important in determining variation in results. Since estrus detection could not be performed continuously over the entire time period that a female was in estrus, most studies reported in Table 1.1 developed a system to estimate the time of estrus onset and the time of estrus conclusion to calculate estrus duration (e.g., if estrus detection was performed every 8 hours, estimated onset of estrus would be time of first detected estrus minus 4 hours). As a result, as the time interval between estrus detection increased, the accuracy of estimates of actual estrus duration decreased.

Six of the studies reported in Table 1.1 gave the time of ovulation as a percentage of estrus, and 3 of these studies reported results for gilts. Time of ovulation as a percentage of estrus in these studies (Almeida et al., 2000; Bracken et al., 2003; and Bortolozzo et al., 2005) ranged from means of 59.5 to 85.0%. These results differ from the Soede and Kemp (1997) assumption that ovulation occurs on average 70% of the way through estrus. Results of the 3

studies completed using sows, however, reported means of 68, 71, and 72% (Soede et al., 1995; Nissen et al., 1997; and Steverink et al., 1997), which in general is in agreement with the claims of Soede and Kemp (1997). This may indicate that utilizing duration of estrus may be more accurate at predicting time of ovulation in sows than in gilts. Further research is necessary, however, to determine the time of ovulation as a percentage of duration of estrus in both gilts and sows, particularly when utilizing this variable as a way to predict the best time of insemination.

Although Soede and Kemp et al. (1997) suggested that duration of estrus is the current best indicator of time of ovulation, the authors also noted that there are several factors that have been found to influence duration of estrus, including presence of boar stimuli, season, stress, and parity. In support of this, Belstra et al. (2004) carried out a large study to evaluate temporal relationships between estrus and ovulation in sows on 3 farms during the spring and summer months. It was reported that both duration of estrus and estrus to ovulation interval appeared to be impacted by parity of the sow, season, and farm. They reported a significant effect of parity on duration of estrus and estrus to ovulation interval, and a linear increase in these 2 variables as parity increased in the summer, but not in the spring. Finally, these authors reported a significant difference in estrus to ovulation interval between 2 genotypes on 1 of the farms evaluated. These results suggest that there is considerable variation in duration of estrus both between and within farms.

In conclusion, a number of studies have suggested that there is a relationship between duration of estrus and time of ovulation in both gilts and sows (Steverink et al., 1997; Almeida et al., 2000; Bortolozzo et al., 2005). It is important to consider, however, that such factors as environment, parity or age, presence of boar stimuli, season, and genetics are likely to have an effect on such measures as duration of estrus and time of ovulation as a percentage of duration of

estrus. Soede and Kemp (1997) recommended that duration of estrus was the current best predictor of time of ovulation and that the time of ovulation as a percentage of duration of estrus is on average 70%, and it appears that this may have some merit when applied to sows. There is more variation, however, in the means reported for the time of ovulation as a percentage of duration of estrus in gilts and, therefore, this approach is probably not a reliable tool to use in gilts. Finally, in both gilts and sows, there was a large degree of individual pig variation in the duration of estrus within a population, as evidenced by studies summarized in Table 1.1. Using 1 number for the time of ovulation as a percentage of estrus for an entire group of females (as suggested by Soede and Kemp [1997]), therefore, will not yield the highest fertility results and, therefore, duration of estrus is probably not a reliable tool to use in predicting time of ovulation. Therefore, management strategies or technologies such as exogenous hormones that can effectively synchronize estrus and ovulation, and render all the previously mentioned factors that cause variation inconsequential would be extremely valuable. Exogenous hormones that could effectively synchronize estrus and/or ovulation in an entire group of females would allow for producers to better predict the onset of estrus and ovulation and inseminate females at an optimal time that will in turn yield the highest fertility results.

Synchronization of estrus

The estrous cycle in swine is composed of 2 phases, namely the follicular phase, which lasts for approximately 5 days, and the luteal phase, which lasts approximately 16 days. At the beginning of the follicular phase, a group of follicles undergoes "recruitment," which is when these selected follicles begin to grow and produce estrogen, a hormone which eventually will become important in the behavioral expression of estrus and will also serve as a feedback response mechanism acting on the hypothalamus. During the follicular phase, these recruited

follicles on the ovary develop in response to follicle stimulating hormone (FSH) and luteinizing hormone (LH) released from the anterior pituitary gland. These two hormones are released in response to positive feedback from gonadotropin releasing hormone (GnRH), which is released from the hypothalamus. While some of the recruited follicles will undergo atresia, other follicles will further develop to a pre-ovulatory stage in which they are capable of ovulating. Pre-ovulatory follicles synthesize sufficient estrogen to induce greater release of LH, and this brings about a pre-ovulatory LH surge, where the concentration of circulating LH in the plasma is greatly increased. This pre-ovulatory LH surge initiates a chain of events which eventually result in ovulation of the pre-ovulatory follicles.

After ovulation, the theca and granulosa cells of ovulated follicles remain on the ovary and develop into the luteal cells of the corpora lutea. The main function of the corpora lutea is to synthesize progesterone throughout the luteal phase of the estrous cycle. Progesterone inhibits follicular growth by serving as a negative feedback mechanism on the release of GnRH. This decrease in circulating GnRH inhibits the growth of follicles to pre-ovulatory stage, thus preventing ovulation. After a period of 12 to 14 days following ovulation, corpora lutea become responsive to prostaglandin F2α (PGF2α), a hormone produced by the endometrium of the uterus that causes luteolysis. Although there is some disagreement as to the exact mechanism by which PGF2α stimulates luteolysis, in general, it is believed that the uterus must be exposed to elevated levels of progesterone before it is able to synthesize and release PGF2α in sufficient quantities (Senger, 2003). Following luteolysis, the resulting decrease in circulating progesterone levels removes the inhibition on the release of GnRH, thus initiating the follicular phase and the chain of events previously described.

Altrenogest

One form of synthetic progesterone, altrenogest, is marketed in the United States under the brand name Matrix® (Merck Animal Health, Summit, NJ). Matrix® has been developed to suppress estrus in gilts and sows, mimicking the mode of action of endogenous progesterone, as previously described. Matrix® is only effective when used in sexually mature gilts that have experienced at least one estrous cycle. In addition, administration does not prevent normal luteolysis, but will continue to inhibit the onset of estrus after luteolysis occurs (Kirkwood, 1999). Matrix® is orally active, and according to the label, the manufacturer recommends administration of 6.8 ml (15 mg altrenogest) per gilt once daily for 14 consecutive days by top-dressing on feed (Merck Animal Health, 2013). When Matrix® is withdrawn after 14 days, the negative inhibition of altrenogest on the release of GnRH is removed and FSH and LH are synthesized and released at higher levels, stimulating further follicular growth and ultimately ovulation. One major advantage of using a product such as Matrix® to synchronize estrus is that females may be in any stage of the estrous cycle at the onset of an altrenogest administration program. For females in the early stages of their estrous cycles, administration of altrenogest coincides with the production of progesterone from the corpora lutea, and at the time of Matrix® withdrawal, circulating endogenous progesterone levels are also declining (via regression of the corpora lutea). For gilts in the middle of their estrous cycle, starting an altrenogest administration program will result in an increased luteal phase length (progesterone levels that will remain elevated) until altrenogest is removed from the feed. Therefore, altrenogest is a tool that can be used to synchronize estrus in an entire group of gilts (Merck Animal Health, 2013).

Altrenogest effects on fertility in gilts

Studies reporting the effect of daily altrenogest administration to gilts on subsequent fertility are summarized in Table 1.2. The 7 papers summarized presented results from 9 studies. Methodology differed between studies particularly with respect to the number of animals involved (which ranged from 13 to 523), the dose of altrenogest administered (12.5 to 20 mg/day), and the duration of altrenogest administered (14 to 19 days). In addition, there were differences between the studies in regards to how estrus detection was performed in the Control groups (i.e., timing of the beginning and duration of estrus detection differed). Finally, it is important to note that the "Control" treatment used in 2 studies (Estienne et al., 2001 and Horsley et al., 2005) consisted of gilts administered altrenogest followed by P.G. 600, whereas the control treatment in the other 7 studies consisted of gilts that were not treated with altrenogest. Therefore, care is needed when interpreting the results of the studies presented in Table 1.2.

In general, the studies summarized in Table 1.2 suggest similar or increased gilt reproductive performance following altrenogest administration. The percentage of gilts exhibiting estrus within 7 to 10 days after withdrawal of altrenogest averaged 87.4% with a range of 67 to 100% and the average interval to estrus after withdrawal of altrenogest was 5.5 days with a range from 4.3 to 7.9 days. It should be mentioned, however, that 2 of the studies reporting the highest intervals to estrus (Davis et al., 1987 and Rhodes et al., 1991) cited problems that could have affected the results. Davis et al. (1987) reported a high incidence of ovulation among gilts not detected to be in behavioral estrus, and Rhodes et al. (1991) used both pre-pubertal and post-pubertal gilts in their trial, and as previously described pre-pubertal gilts may have a more irregular first estrous cycle (Eliasson, 1989).

Only 2 studies reported pregnancy rates of gilts administered altrenogest, and the average of these studies was 88.9% (range 88.5 to 89.3%). Across the 4 studies reporting farrowing rates, the average was 77.4% (range 64 to 88.4%), and 3 of the 4 studies reported a higher farrowing rate in gilts administered altrenogest compared to control gilts not administered the product. Finally, the total number of piglets born and born alive per litter averaged 10.0 and 8.9 respectively (range 9.6 to 10.6 pigs and 8.5 to 9.2 pigs, respectively). When compared to control treatments, total piglets born and born alive per litter were greater by between 1.0 and 13.8% for gilts treated with altrenogest. This increase could be attributed to an increased ovulation rate in gilts administered altrenogest prior to mating, as reported by Davis et al. (1979, 2 studies), Ashworth et al. (1992), and Martinet-Botte et al. (1995). These authors reported that gilts administered altrenogest had an increase of between 5.5 and 36.0% in the number of follicles ovulated compared to gilts not administered altrenogest.

In conclusion, the results of the studies evaluating the effect of altrenogest administration in gilts are quite variable, potentially due to environmental, nutritional, genetic, and management differences between studies. In addition, there were significant differences in the methodology used between many of the studies which could have affected results. Despite these differences, the results summarized in Table 1.2 appear to report similar or favorable results in fertility for gilts treated with altrenogest compared to untreated controls.

Effect of duration of altrenogest administration on fertility in gilts

Only 2 studies have compared differences in duration of altrenogest administration on fertility results in gilts and these studies are summarized in Table 1.3. It should be noted that there was a considerably smaller number of animals used by Webel (1978) and this author did not report which day post altrenogest withdrawal was used as the time of measurement of the

percentage of gilts in estrus, therefore, care should be used when comparing the results of the two studies (Webel, 1978 and Stevenson and Davis, 1982).

In general, the results reported in Table 1.3 suggest that gilts administered altrenogest for 18 days had higher fertility compared to gilts administered altrenogest for 14 days. In addition to the results summarized in Table 1.3, Stevenson and Davis (1982) reported that the range in days from end of altrenogest administration to estrus was greater for gilts administered altrenogest for 14 days compared to 18 days. The results of Webel (1978) are in agreement with these findings, reporting a lower standard deviation around the mean for days from the end of altrenogest administration to estrus for gilts administered altrenogest for 18 days compared to 14 days (0.89 versus 1.36 days, respectively). In addition, Stevenson and Davis (1982) reported that more gilts administered altrenogest for 18 days were in estrus on the peak day of the onset of estrus (57.1 versus 34.6%), however, these authors also reported that there was no difference in the number of gilts in estrus observed between feeding duration treatments 10 days after altrenogest withdrawal. Also, although Stevenson and Davis (1982) reported that there was no difference in farrowing rate between gilts that were administered altrenogest for 18 compared to 14 days, the authors did report an increase (~6%) in the total number of piglets born and number of piglets born alive per litter for gilts administered altrenogest for the longer time period (Table 1.3).

In conclusion, the results of the 2 studies summarized in Table 1.3 evaluating the duration of altrenogest administration on fertility in gilts suggest that altrenogest administration for 18 days compared to 14 days is more effective in synchronizing estrus. However, there is a very limited amount of published literature evaluating the duration of altrenogest administration in gilts. Further research in this area is necessary, therefore, and particularly research on the effect of length of altrenogest administration on timing of ovulation, as there is currently no reported

data on this measure. According to the product label, however, the use of Matrix® for greater than 14 days is prohibited, therefore, further research is on the effect of length of altrenogest administration (greater than 14 days) is difficult.

Synchronization of Ovulation

Many studies have been completed examining the effect of exogenous hormones on the synchronization of ovulation in pigs. Synthetic exogenous hormones that have been evaluated include GnRH agonists (Webel, 1978; Brussow et al., 1996; Martinet-Botte et al., 2010; Wongkaweewit et al., 2011), luteinizing hormone (do Lago et al., 2005; Degenstein et al., 2008), and human chorionic gonadotropin (Webel, 1978; Brussow et al., 1990; Horsley et al., 2005; Degenstein et al., 2008; Kaeoket, 2008; Manjarin et al., 2010; Wongkaweewit et al., 2011). These studies have reported varying levels of success in terms of synchronization of ovulation and varying results in terms of subsequent fertility compared to untreated sows. Despite promising results reported by some of these studies in terms of synchronization of ovulation and fertility, many of these synthetic hormones are not commercially available in the U.S., either because they are not approved or due to problems associated with obtaining and marketing these hormones.

OvuGel®

Triptorelin acetate is a synthetic GnRH agonist which mimics the role of endogenous GnRH by stimulating the anterior pituitary gland to release a surge of LH which, as previously described, is a precursor to ovulation. Triptorelin acetate is currently approved for use in sows and is marketed in the United States under the product name OvuGel® (JBS United, Sheridan, IN). According to the product label, 2 ml (200 µg triptorelin acetate) of OvuGel® is

administered intra-vaginally 96 hours after weaning, and ovulation will occur 40 to 48 hours later. It is recommended that a single insemination is performed 22 hours after the time of administration of OvuGel®. The primary goal of utilizing OvuGel® is to synchronize ovulation in an entire group of females, allowing for one single insemination given to each female, regardless of whether that animal is exhibiting behavioral signs of estrus or not.

Effect of OvuGel® on fertility in sows

Studies reporting the effect of OvuGel® on reproductive performance in sows are summarized in Table 1.4. It is important to note that traditional pregnancy and farrowing rates are calculated as the number pregnant/farrowing divided by the number inseminated, however, all sows allotted to receive OvuGel® were inseminated in each of the studies; whereas only sows allotted to the control group that expressed behavioral estrus where inseminated. Therefore, in this section, the number of sows pregnant or farrowing divided by the number of sows that were allotted to receive each treatment (OvuGel® or Control) is used to calculate pregnancy and farrowing rates.

In general, the 7 studies summarized in Table 1.4 report similar reproductive performance for sows administered OvuGel® compared to control sows, and there were no statistically significant differences reported for pregnancy and farrowing rates or total number of piglets born and born alive. Additionally, it is important to mention that there were differences in the methodology used between studies regarding the numbers of animals used, amount of OvuGel® administered to sows, and the timing and number of inseminations given to sows following OvuGel® administration. Therefore, care is needed when interpreting the results of the studies presented in Table 1.4.

Few studies have been completed reporting the effect of OvuGel® administration on the timing of ovulation or on the percentage of sows that have ovulated within 2 to 3 days after OvuGel® administration (the time at which ovulation should occur after OvuGel® administration). Of the studies reporting time of ovulation in Table 1.4, Taibl et al. (2008) reported that 78.2% of sows administered OvuGel® ovulated within 48 hours of administration, compared to only 45.8% of control sows that ovulated in that same time period. Furthermore, Knox et al. (2011) reported that sows administered OvuGel® ovulated sooner after the onset of estrus than control sows (34.6 versus 41.5 hours, respectively). These results suggest that OvuGel® administration advances the time of ovulation in sows.

Three studies summarized in Table 1.4 reported the effect of OvuGel® administration on pregnancy rates and averaged across studies the means were 77.6% (range of 73.3 to 84.3%) for OvuGel® administered sows compared to 78.9% (range of 73.8 to 80.6%) for control sows.

Of the 7 studies reporting farrowing rate for sows administered OvuGel®, the mean averaged across studies was 72.9% (range of 56.3 to 81.0%) compared to 72.2% (range of 56.4 to 83.3%) for control sows. Although Taibl et al. (2008) reported a 13.0% increase in farrowing rate for sows administered OvuGel® compared to control sows and Knox et al. (2011) reported a 12.8% decrease in farrowing rate for sows administered OvuGel®, both studies used a considerably smaller number of animals (total number of sows $\leq$ 83) than the other studies summarized in Table 1.4 and neither study reported the results to be statistically significant.

Four studies summarized in Table 1.4 reported total number of piglets born and 7 studies reported number of piglets born alive. Six of the studies summarized in Table 1.4 reported that the difference between treatments in total number of piglets born and born alive was within 0.5 piglets. In contrast to this, however, Knox et al. (2011) reported that sows administered

OvuGel® had 1.1 higher total number of piglets born and Taibl et al. (2008) reported a decrease of 0.8 piglets born alive for sows administered OvuGel® compared to control sows. It should be noted, however, that neither of these treatment differences were statistically significant, and as mentioned previously a smaller number of animals were used in both studies compared to the other studies summarized in Table 1.4.

Finally, 3 studies reporting the effect of OvuGel® administration on piglet index are summarized in Table 1.4. Piglet index is defined as the number of piglets born alive divided by the number of sows allotted to a treatment times 100, and is an important measure to consider when evaluating fertility results for sows administered OvuGel®. As previously described, all sows receiving OvuGel® were inseminated regardless of whether or not they were exhibiting physical signs of estrus at insemination, whereas control sows were only inseminated if they expressed behavioral estrus. Two of the 3 studies summarized in Table 1.4, both carried out by Knox et al. (2011), reported a lower piglet index for sows administered OvuGel® compared to control sows. Johnston et al. (2013), however, reported an increase in piglet index for sows administered OvuGel® compared to control sows.

Effect of OvuGel® on fertility in gilts

There have been no published studies evaluating the effect of OvuGel® on either the timing of estrus and ovulation or on reproductive performance in gilts. Three unpublished studies (Johnston et al., 2013a, b, and c), however, are presented in Tables 1.5 and 1.6 which report on the effect of OvuGel® administration on the time of first estrus expression, pregnancy and farrowing rates, and litter size. Each of these studies involved the use of OvuGel® after a 14 d administration period of Matrix® to synchronize estrus in the gilts, however, it is important to note that gilts were only allotted to the studies and started on Matrix® if they were between days

4 and 16 of their estrous cycle (with day 0 equating to the first day of estrus expression). OvuGel® was administered 5 days after the last feeding of Matrix® and gilts were inseminated once 24 hours later, regardless of the expression of behavioral estrus. Gilts on the Control treatment were inseminated on the first morning that behavioral estrus was detected and subsequently once on each morning that they remained in estrus. Similar to the pregnancy and farrowing rate calculations used for sows (as previously discussed), the pregnancy and farrowing rates for gilts were calculated as the number of gilts pregnant or farrowing divided by the number of gilts that were allotted to receive each treatment (OvuGel® or Control). Finally, it must be noted that no statistical analysis was carried out for the data summarized in Table 1.5 (day of first estrus expression); therefore, only numeric differences between treatments can be discussed (statistical analysis was completed for pregnancy and farrowing rates and litter size; summarized in Table 1.6).

Day of first estrus expression Three unpublished studies that evaluated the effect of OvuGel® administration after the end of Matrix® administration on the day of first estrus expression are summarized in Table 1.5. In general, despite the same methodology being used, there was variation between the 3 studies both in terms of day of onset of estrus following the last feeding of Matrix® and for percentage of animals not expressing estrus within the time frame evaluated (1 to 14 days after last feeding of Matrix®; Table 1.5). In all 3 studies, the majority (range of 50.0 to 85.8%) of both control gilts and OvuGel® administered gilts expressed estrus on either day 6 or 7 following the last feeding of Matrix®. Across studies, a higher percentage of OvuGel® administered gilts were in estrus on day 6 following the last feeding of Matrix® compared to Control gilts (47.5 vs. 39.4% for OvuGel® treated and control gilts, respectively), however, a lower percentage of OvuGel® administered gilts were in estrus on day 7 following the

last feeding of Matrix® compared to Control gilts (14.4 vs. 29.2%, respectively). Finally, across all 3 studies, the range in the number of gilts not expressing estrus was greater for gilts administered OvuGel® (14.8 to 41.0%, respectively) compared to the Control (3.0 to 18.2%, respectively).

Pregnancy and farrowing rates The 3 unpublished studies summarizing the effect of OvuGel® administration on pregnancy and farrowing rates and litter size are summarized in Table 1.6. Only Control gilts which had exhibited estrus and were inseminated within 8 days after the end of Matrix® administration were included in the analysis for pregnancy and farrowing rates and litter size. In general, there was a high degree of variation in the pregnancy rates for Control gilts across studies (range from 73.0 to 91.5%), while the pregnancy rates for gilts administered OvuGel® had a smaller range across studies (76.7 to 83.1%). One of the studies reported that Control gilts had a higher ($P \leq 0.05$) pregnancy rate compared to gilts administered OvuGel® (91.5 and 82.5% for Control and gilts administered OvuGel®, respectively). In contrast to this study, however, a second study reported that Control gilts had a lower ($P \leq 0.05$) pregnancy rate compared to gilts administered OvuGel® (73.0 and 83.1% for Control and gilts administered OvuGel®, respectively), whereas, the third study by Johnston et al. (2013a, b, and c) reported no difference ($P > 0.05$) in pregnancy rates between treatments (Table 1.6).

Similar to pregnancy rate, there was a larger range in the farrowing rates across studies for Control gilts (71.6 to 89.3%) compared to gilts administered OvuGel® (67.9 to 78.6%). Two of the studies by Johnston et al. (2013a, b, and c) reported no effect ($P > 0.05$) of OvuGel® administration on farrowing rate, however, the third study reported a higher ($P \leq 0.05$) farrowing

rate for Control gilts compared to gilts administered OvuGel®(89.3 and 78.6% for the Control and gilts administered OvuGel®, respectively).

There is disagreement in the findings for the effect of OvuGel® administration in gilts on pregnancy and farrowing rates between the 3 studies by Johnston et al. (2013a, b, and c), and from the data that was collected in these studies it may be difficult to fully understand the pregnancy and farrowing rate results that were obtained.

The key to achieving high fertility after OvuGel® administration and a single fixed-time insemination 24 hours later is that ideally administration should occur at a time when there are mature follicles on the ovary that are capable of responding to either a naturally occurring LH surge or one induced by OvuGel® administration, and, that ovulation should occur within 40 to 48 hours of OvuGel® administration. Ultrasounding was not carried out in these studies, and, therefore, it is impossible to determine any relationship between follicular maturity and time of ovulation and the pregnancy rates obtained in these studies. However, if follicles were not capable of responding to the LH surge at the time of OvuGel® administration, fertilization would not have occurred, leading to a lower pregnancy rate overall. In addition, ovulation should have occurred on either day 6 or 7 after the end of Matrix® administration (OvuGel® was administered on day 5; according to the OvuGel® product label ovulation should occur 40 to 48 hours after OvuGel® administration), however, the time of ovulation was not measured in any of the 3 studies. Although the day of estrus onset was measured in these studies, as previously discussed, using the duration of estrus may not be a reliable indicator for time of ovulation, particularly in gilts. There is more variation in the time interval to ovulation as a percentage of estrus duration for gilts (as summarized in Table 1.1), therefore, using 1 number for the time of ovulation for an entire group of females may not yield high fertility results.

Litter size Two of the unpublished studies by Johnston et al. (2013a, b, and c) reported no effect ($P > 0.05$) of OvuGel® administration on total number of piglets born per litter or the number of piglets born alive per litter. There was a trend ($P = 0.07$), however, for Control gilts in one of those studies (Johnston et al., 2013b) to have a higher total number of piglets born per litter (15.0 and 13.9 for the Control and gilts administered OvuGel®, respectively). In contrast to the 2 aforementioned studies, however, the third study reported that Control gilts had a higher ($P \leq 0.05$) total number of piglets born and born alive per litter (15.6 and 14.6, and 13.2 and 12.6 for total number of piglets born and born alive per litter for Control gilts and gilts administered OvuGel®, respectively).

In summary, a significant number of studies have been conducted evaluating the effect of OvuGel® administration on fertility measures in sows, and in general the results of these studies suggest that OvuGel® is effective in synchronizing ovulation in a group of sows and yields similar fertility results compared to sows not administered OvuGel®. There are no published studies, however, evaluating the effect of OvuGel® administration in gilts on the timing of ovulation or fertility measures. Three unpublished studies evaluating the use of this product in gilts reported differing fertility results, however, it is difficult to determine the reason for the differing results obtained across studies because no ultrasounding was conducted to determine when ovulation occurred. The timing of ovulation in relation to the timing of OvuGel® administration and insemination is crucial in successfully utilizing OvuGel® and a fixed-time insemination program. Further research is needed; therefore, on the effect of OvuGel® administration in gilts and its effect on the timing of ovulation and fertility measures.

Conclusion

Based on the literature review presented in this chapter it is well established that insemination must occur within a certain time frame relative to ovulation (24 hours prior to ovulation to 4 hours after ovulation) for the highest fertility rates to be obtained (Waberski et al., 1994; Nissen et al., 1995; Soede et al., 1995; Steverink et al., 1997; Bracken et al., 2003), however, there is no practical way currently to determine the time of ovulation in gilts.

Altrenogest is widely used in the swine industry as an effective method of synchronizing estrus in both gilts and sows; however, the effect of Matrix® on time of ovulation is not well understood. In addition, administering OvuGel® has been shown to effectively synchronize ovulation in a group of sows, which ultimately allows for a single fixed-time insemination. Little is known, however, of the effect of OvuGel® administration on the timing of ovulation or fertility results in gilts. In theory, the use of OvuGel® in a group of weaned sows is a simpler strategy than in gilts because the sows should be at a similar stage of estrous cycle. Determining how to properly utilize OvuGel® in gilts is more complex, however, because in typical commercial practice, gilts within a group are in estrus and ovulating at different times, and therefore, it is difficult to predict time of estrus and ovulation across a group. Matrix® administration could serve to synchronize estrus in a group of gilts, thus allowing for subsequent administration of OvuGel® and a single fixed-time insemination. Therefore, further research is necessary to understand 1) the effects of OvuGel® administration on reproductive performance in gilts, and 2) the effect of timing of OvuGel® administration on reproductive performance in gilts. The results of this research would greatly benefit producers in terms of understanding how to most effectively utilize programs such as Matrix® and OvuGel®, to allow for greater efficiency of artificial insemination.

LITERATURE CITED

Almeida, F. R. C. L., S. Novak, G. R. Foxcroft. 2000. The time of ovulation in relation to estrus duration in gilts. Therio. 53: 1389-1396.

Ashworth, C. J., C. S. Haley, and I. Wilmut. 1992. Effect of Regumate on ovulation rate, embryo survival and conceptus growth in Meishan and Landrace X large white gilts. Therio. 37:433-443.

Augspurger, N. R., M. E. Johnston, M. E. Swanson, and S. K. Webel. 2012. Single and double, fixed-time insemination of postpartum sows given intravaginal triptorelin gel. J. Anim. Sci. Vol. 90. (Suppl. 3):731.

Bortolozzo, P. F., D. A. Uemoto, P. E. Bennemann, W. Barioni Jr., and I. Wentz. 2005. Influence of time of insemination relative to ovulation and frequency of insemination on gilt fertility. Therio. 64: 1956-1962.

Brussow, K. P., J. Ratky, W. Kanitz, and F. Becker. 1990. Determination of the duration of ovulation in gilts by means of laparoscopy. Reprod. Dom. Anim. 25: 184-190.

Brussow, K .P., W. Jochle, and U. Huhn. 1996. Control of ovulation with a GnRH analog in gilts and sows. Therio. 46: 625-934.

Brussow, K. P., and M. Wahner. 2011. Biological and technological background of estrus synchronization and fixed-time ovulation induction in the pig. Biotech. Anim. Husbandry. 27: 533-545.

Bracken, C. J., T. J. Safranski, T. C. Cantley, M. C. Lucy, and W. R. Lamberson. 2003. Effect of time of ovulation and sperm concentration on fertilization rate in gilts. Therio. 60: 669-676.

Belstra, B. A., W. L. Flowers, and M. T. See. 2004. Factors affecting temporal relationships between estrus and ovulation in commercial sow farms. Anim. Repro. Sci. 84: 377-394.

Davis, D. L., J. W. Knight, D. B. Killian and B. N. Day. 1979. Control of estrus in gilts with a progestogen. J. Anim. Sci. 49: 1506-1509.

Davis, D. L., J. S. Stevenson, D. S. Pollman and G. L. Allee. 1987. Estrous and litter traits in gilts altered by altrenogest, flushing, and pubertal status. J. Anim. Sci. 64: 1117-1126.

Degenstein, K. L, R. O'Donoghue, J. L. Patterson, E. Beltranena, D. J. Ambrose, G. R. Foxcroft, M.K. Dyck. 2008. Synchronization of ovulation in cyclic gilts with porcine luteinizing hormone and its effects on reproductive function. Therio. 70: 1075-1085.

DeJarnette, J. M., R. G. Saacke, J. Bame, and C. J. Vogler. 1992. Accessory sperm: their importance to fertility and embryo quality, and attempts to alter their numbers in artificially inseminated cattle. J. Anim. Sci. 70: 484-491.

do Lago, V., W. L. Vianna, R. D. Gama, A. de Campos Rosseto, M. E. Pinese, and A. de Sant'Anna Moretti. 2005. Second oestrus synchronization and precocious embryo viability after puberty induction in gilts by the use of gonadotropin treatment. Reprod. Dom. Anim. 40: 141-144.

Eliasson, L. 1989. A study on puberty and oestrus in gilts. J. Vet. Med. 36: 46-54.

Estienne, M. J., A. F. Harper, B. R. Horsley, C. E. Estienne, and J. W. Knight. 2001. Effects of P.G. 600 on the onset of estrus and ovulation rate in gilts treated with Regu-mate. J Anim. Sci. 79: 2757-61.

Flowers, W. L., M. E. Johnston, S. K. Webel, and M. E. Swanson. 2013. Effectiveness of OvuGel® for induction of ovulation in a single, fixed-timed insemination program for weaned sows. p. 124 in Proc. of the ASAS Midwestern Section Annual Mtg. Des Moines, IA.

Horsley, B. R., M. J. Estienne, A. F. Harper, S. H. Purcell, H. K. Baitis, W. E. Beal, and J. W. Knight. 2005. Effect of P.G. 600 on the timing of ovulation in gilts treated with altrenogest. J. Anim. Sci. 83: 1690-169.

Hunter, R. H. F. 1981. Sperm transport and reservoirs in the pig oviduct in relation to the time of ovulation. J. Reprod. Fertil. 63: 109-117.

Johnston, M. E., A. M. Gaines, M. E. Swanson, and S. K. Webel. 2009. Timed insemination following GnRH agonist administration in weaned sows. p. 170 in the Proc. of the VIII Internat. Conf. on Pig Reprod. Banff Canada.

Johnston, M. E., M. E. Swanson, and S. K. Webel. 2013. Timed insemination following OvuGel® administration in weaned sows. p. 49 in the Proc. of the ASAS Midwestern Section Annual Mtg. Des Moines, IA.

Johnston, M. E., M. E. Swanson, and S. K. Webel. 2013a, b and c. (unpublished raw data).

Kaeoket, K. 2008. Study on the oestrous synchronization in gilts by using progestin altrenogest and hCG: Its effect on the follicular development, ovulation time, and subsequent reproductive performance. Reprod. Dom. Anim. 43: 127-129.

Kemp, B. and N. M. Soede. 1997. Consequences of variation in interval from insemination to ovulation on fertilization in pigs. J. Reprod. Fertil. (Suppl. 52): 79-889.

Kirkwood, R. N. 1999. Pharmacological intervention in swine reproduction. J. Swine. Health Prod. 7: 29-35.

Knox, R. V., K. L. Willenburg, S. L Rodriguez-Zas, D. L. Greger, H. D. Hafs, and M. E. Swanson. 2011. Synchronization of ovulation and fertility in weaned sows treated with intravaginal triptorelin is influenced by timing of administration and follicle size. Therio. 75: 308-319.

Kraeling. R., R. Knox, J. Taibl, M. Johnston, M. Swanson, and S. Webel. 2013. Effect of triptorelin administered intravaginally as OvuGel® on the timing of ovulation in mature gilts after estrus synchronization with Matrix. p. 391 in Proc. of the AASV 44th Annual Mtg. San Diego, CA.

Manjarin, R., J. C. Garcia, J. C. Dominguez, M. J. Castro, B. Alegre, J. D. Munoz, and R. N. Kirkwood. 2010. Effect of gonadotropin treatment on estrus, ovulation, and litter size in weaned and anestrous sows. J. Anim. Sci. 88: 2356-2360.

Martinette-Botte, F., F. Bariteau, Y. Forgerit, C. Macar, A. Moreau, M. Terqui, and J. P. Signoret. 1990. Control of oestrus in gilts II. Synchronization of oestrus with a progestagen, altrenogest (Regumate): Effect on fertility and litter size. Anim. Reprod. Sci. 22: 227-233.

Martinette-Botte, F., F. Bariteau, Y. Forgerit, C. Macar, P. Poirier, and M. Terqui. 1995. Synchronization of oestrus in gilts with altrenogest: effects on ovulation rate and foetal survival. Anim. Reprod. Sci. 39: 267-274.

Martinette-Botte, F., E. Venturi, P. Guilloet, M. A. Driancourt, and M. Terqui. 2010. Induction and synchronization of ovulations of nulliparous and multiparous sows with an injection of gonadotropin-releasing hormone agonist (Receptal). Therio. 73: 332-342.

Nissen, A. K., N. M. Soede, P. Hyttel, M. Schmidt, and L. D'Hoore. 1997. The influence of time of insemination relative to time of ovulation on farrowing frequency and litter size in sows, as investigated by ultrasonography. Therio. 47:1571-1582.

Pollard, J. W., C. Plante, W. A. King, P. J. Hansen, K. J. Betteridge, S. S. Suárez. 1991. Fertilizing capacity of bovine sperm may be maintained by binding to oviductal epithelial cells. Biol. Reprod. 44: 102-107.

Pursel, V. G, Schulman, L. L. and Johnson, L. A. 1978. Distribution and morphology of fresh and frozen–thawed sperm in the reproductive tract of gilts after insemination. Biol. Reprod. 19: 69-76.

Raychoudhury, S. S., S. S. Suárez. 1991. Porcine sperm binding to oviductal explants in culture. Therio. 36: 1059-1070.

Rhodes, M. T., D. L. Davis and J. S. Stevenson. 1991. Flushing and altrenogest affect litter traits in gilts. J. Anim. Sci. 69:34-40.

Saacke, R. G., S. Nadir, and R. L. Nebel. 1994. Relationship of semen quality to sperm transport, fertilization and embryo quality in ruminants. Therio. 41: 45-49.

Senger, P. L. 1997. Pathways to pregnancy and parturition. 2nd Edition. Cadmus Professional Communications. Ephrata, PA.

Soede, N. M., C. C. H. Wetzels, W. Zonda, M. A. I. de Koning and B. Kemp. 1995. Effects of time of insemination relative to ovulation, as determined by ultrasonography, on fertilization rate and accessory sperm count in sows. J. Reprod. Fertil. 104: 99-106.

Soede, N. M. and B. Kemp. 1997. Expression of oestrus and timing of ovulation in pigs. J. Reprod. Fertil. (Suppl. 52): 91-103.

Stevenson, J. S., and D. L. Davis. 1982. Estrous synchronization and fertility in gilts after 14- or 18- day feeding of altrenogest beginning at estrus or diestrus. J. Anim. Sci. 55: 119-123.

Steverink, D. W. B., N. M. Soede, E. G. Bouwan and B. Kemp. 1997. Influence of insemination-ovulation interval and sperm cell dose on fertilization in sows. J. Reprod. Fert. 111: 165-171.

Steverink, D. W. B., N. M. Soede, G. J. R. Groenland, F. W. van Schie, J. P. T. M. Noordhuizen, and B. Kemp. 1999. Duration of estrus in relation to reproduction results in pigs on commercial farms. J. Anim. Sci. 77: 801-809.

Taibl, J. N., S. M. Breen, S. K. Webel, and R. V. Knox. 2008. Induction of ovulation using a GnRH agonist for use with fixed time AI in weaned sows. Theriol. 70: 1400.

Taibl, J. N., S. M. Breen, S. K. Webel, M. E. Swanson, and R. V. Knox., 2009. Effect of synchronizing ovulation in weaned sows using OvuGel® with single fixed time AI on pregnancy rate and litter size. p. 160 in the Proc. of the VIII International Conf. on Pig Reprod. Banff Canada.

Waberski, D., K. F. Weitze, T. Gleumes, M. Schwarz, T. Willmen and R. Petzoldt. 1994. Effect of time of insemination relative to ovulation on fertility with liquid and frozen boar semen. Therio. 42: 831-840.

Webel, S. 1978. Ovulation control in the pig. In: D. B. Crighton, N. B. Haynes, G. R. Foxcroft and G. E. Lamming (Ed.) Control of ovulation. pp. 421-434. Butterworths, London.

Weitze, K. F., H. Wagner-Rietschel, D. Waberski, L. Richter, and J. Krieter. 1988. The onset of heat after weaning, heat duration, and ovulation as major factors in AI timing in sows. Reprod. Dom. Anim. 29: 433-443.

Wongkaweewit, K., P. Prommachart, R. Raksasub, K. Buranamnuay, M. Techakumphu, F. De Rensis, P. Tummaruk. 2011. Effect of the administration of GnRH or hCG on time of

ovulation and the onset of estrus-to-ovulation interval in sows in Thailand. Trop. Anim. Prod. 44: 467-470.

Yeske, P, M. Johnston, M. Swanson, S. Webel and R. Kraeling. 2013. Timed insemination following intravaginal OvuGel® treatment in postpartum sows. p. 45 in Proc. of the AASV44th Annual Mtg. San Diego, CA.

TABLES

Table 1.1. Summary of studies characterizing the duration of estrus, onset of estrus to ovulation, and time of ovulation as a percentage of estrus in gilts and sows.

Item	# Pigs	Gilts	Post-pubertal	Frequency of Estrus Detection (h)[1]	Frequency of Ultrasonography (h)[2]	Mean / St.Dev.	Range
Estrus duration, h							
Eliasson, 1989[3]	149	Y	Y	12	.	50.1	.
Weitze et al., 1994	483	N	NA	8	8	59.6 ± 14.8	.
Soede et al., 1995	151	N	NA	8	4	50.0	24-88
Steverink et al., 1997	115	N	NA	8	4	59.0 ± 12	24-88
Steverink et al., 1999[4,5]	2180	Y	.	12	.	41.2 ± 1.0	19-52
Steverink et al., 1999[4]	11246	N	NA	12	.	50.1 ±1.1	32-69
Almeida et al., 2000	92	Y	Y	6	6	52.6 ± 8.56	30-72
Belstra et al., 2004[6]	501	N	NA	6	6	55.2	.
Bortolozzo et al., 2005	102	Y	N	8	8	50.0 ± 11	24-72
Onset of estrus to ovulation, h							
Weitze et al., 1994	427	N	NA	8	8	44.6 ±12.8	.
Soede et al., 1995	151	N	NA	8	4	35.0	10-58
Almeida et al., 2000	92	Y	Y	6	6	43.9 ± 6.23	30-60
Knox et al., 2001	174	N	Y	.	.	40.6	.
Bracken et al., 2003	59	Y	Y	12	6	33.5 ± 1.6	.
Belstra et al., 2004[5]	501	N	Y	6	6	41.8	.
Bortolozzo et al., 2005	102	Y	N	.	.	34.0 ± 9	16-56
Time of ovulation (as % of estrus)							
Soede et al., 1995	151	N	NA	8	4	72.0	39-133
Nissen et al., 1997	143	N	NA	8	6	71 ± 14	.
Steverink et al., 1997	115	N	NA	8	4	68.0 ± 10	.
Almeida et al., 2000	92	Y	Y	6	6	85.7 ± 13.85	60-138
Bracken et al., 2003	59	Y	Y	12	6	59.5 ± 2.0	.
Bortolozzo et al., 2005	102	Y	N	8	8	68.0	.

[1]Estrus detection performed with the use of a "teaser" boar and the back pressure test.

[2]Time of ovulation was determined by trans-rectal or trans-abdominal ultrasonography.

[3]Mean estrus length of second observed estrus in gilts.

[4]Results from 55 commercial farms.

[5]Pubertal status of gilts was not reported.

[6]Mean generated from the mean estrus duration from 3 farms at 2 time periods for each farm.

Table 1.2. Summary of studies evaluating the effect of altrenogest in gilts on percentage of gilts displaying estrus, mean interval to estrus, pregnancy rate, farrowing rate, total number of piglets born, and number of piglets born alive.

Item	# Pigs	Post-pubertal	Dose of altrenogest (mg/day)	Duration of administration (d)	Control	Altrenogest	*P*-value
Percentage of gilts displaying estrus ≤ 7 d							
Davis et al., 1979[1]	25	Y	12.5	19	100.0[a]	80.0[b]	< 0.05
Davis et al., 1979[1]	13	Y	12.5	18	100.0	100.0	> 0.05
Davis et al., 1987[1]	28	Y	15	14	55.0[b]	67.0[a]	< 0.05
Davis et al., 1987[1,2]	91	Y	15	14	67.0	71.0	> 0.05
Martinette-Botte et al., 1990	523	Y	20	18	97.3	95.9	.
Martinette-Botte et al., 1995	227	Y	20	18	93.0	93.0	.
Estienne et al., 2001[3]	58	Y	15	18	82.7	89.7	> 0.05
Horsley et al., 2005[3]	50	Y	15	18	100.0	100.0	> 0.05
Percentage of gilts displaying estrus ≤ 10 d							
Rhodes et al., 1991	267	N	15	14	67.0[b]	90.0[a]	< 0.05
Interval to estrus after withdrawal of altrenogest, d							
Davis et al., 1979[1]	25	Y	12.5	19	.	4.9 ± 1.1	.
Davis et al., 1979[1]	13	Y	12.5	18	.	4.5 ± 0.7	.
Davis et al., 1987[1]	28	Y	15	14	7.1	7.9	> 0.05
Davis et al., 1987[1, 2]	91	Y	15	14	13.7	5.7	> 0.05
Rhodes et al., 1991	267	N	15	14	7.4 ± 0.2	7.0 ± 0.2	.
Estienne et al., 2001[3]	58	Y	15	18	4.0 ± 0.1	4.2 ± 0.1	> 0.05
Horsley et al., 2005[3]	50	Y	15	18	4.1[b]	4.6[a]	0.01
Pregnancy rate, %							
Martinette-Botte et al., 1995	227	Y	20	18	77.4[b]	89.3[a]	< 0.05
Estienne et al., 2001[3]	58	Y	15	18	91.7	88.5	> 0.05
Farrowing rate, %							
Davis et al., 1987[1]	28	Y	15	14	83.0	78.0	> 0.05
Davis et al., 1987[1, 2]	91	Y	15	14	59.0	64.0	> 0.05
Martinette-Botte et al., 1990	523	Y	20	18	80.8[b]	88.4[a]	< 0.05
Rhodes et al., 1991	267	N	15	14	76 .0[b]	79.0[a]	< 0.05
Total number of piglets born							
Davis et al., 1987[1]	28	Y	15	14	9.8	10.6	> 0.05
Davis et al., 1987[1, 2]	91	Y	15	14	8.7	9.9	0.06
Martinette-Botte et al., 1990	523	Y	20	18	9.1 ± 2.3[b]	9.6 ± 2.3[a]	< 0.05
Rhodes et al., 1991	267	N	15	14	9.6 ± 0.4	9.7 ± 0.3	> 0.05
Number of piglets born alive							
Davis et al., 1987[1]	28	Y	15	14	8.4	8.5	> 0.05
Davis et al., 1987[1, 2]	91	Y	15	14	8.4	9.2	> 0.05
Martinette-Botte et al., 1990	523	Y	20	18	8.5[b]	9.1[a]	< 0.02
Rhodes et al., 1991	267	N	15	14	8.5 ± 0.4[b]	8.8 ± 0.3[a]	< 0.05

[a,b]Means within a row with different superscripts differ ($P < 0.05$).

[1]Results of two trials reported in the same publication.

[2]Pubertal status of gilts not known.

[3]Control group was administered P. G. 600 24 h after altrenogest withdrawal.

Table 1.3. Summary of studies evaluating the effect of duration of altrenogest administration on percentage of gilts displaying estrus, mean interval to estrus, farrowing rate, total number of pigs born, number of pigs born alive in gilts.

					Duration of Altrenogest Administration		
Item	# Pigs	Gilts	Post-pubertal	mg altrenogest/day	14	18	*P*-value
Percentage of gilts displaying estrus ≤ 6 d							
Webel, 1978[1]	35	Y	Y	12.5	89.0	94.0	.
Stevenson and Davis, 1982	160	Y	Y	15	89.0	96.0	
Interval to estrus after withdrawal of altrenogest, d							
Webel, 1978	35	Y	Y	12.5	5.3	3.6	.
Stevenson and Davis, 1982	160	Y	Y	15	5.3	5.4	> 0.05
Farrowing rate, %							
Stevenson and Davis, 1982	160	Y	Y	15	79.8	79.2	> 0.05
Total number of piglets born							
Stevenson and Davis, 1982	160	Y	Y	15	10.0	10.6	> 0.05
Number of piglets born alive							
Stevenson and Davis, 1982	160	Y	Y	15	9.3	10.0	> 0.05

[1]Day by which the measurement of the percentage of gilts in estrus was not reported.

Table 1.4. Summary of studies evaluating the effect of OvuGel® on reproductive measures in sows.

Item	# Pigs	Amount of OvuGel® administered (µg)	Time of Insemination following OvuGel® administration (h)	Timing of OvuGel® administration (d) following weaning	Number semen doses given to OvuGel® sows	Control	OvuGel®	*P* - value
Percentage of sows ovulating ≤ 2 d								
Taibl et al., 2008	73	200	Estrus onset	96	2	45.8[b]	78.2[a]	< 0.001
Percentage of sows in estrus at AI								
Johnston et al., 2009[1]	300	.	24	96	1	100.0[a]	84.0[b]	0.001
Interval from estrus to ovulation, h								
Knox et al., 2011[2]	83	100	8, 32	96	2	41.5[a]	34.6[b]	0.02
Pregnancy rate, %[3]								
Knox et al., 2011[2]	83	100	8, 32	96	2	80.6	73.3	0.71
Tiabl et al., 2008	73	200	Estrus onset	96	2	73.8	75.2	0.74
Yeske et al., 2013	397	200	21-22	96	1	82.3	84.3	0.61
Farrowing rate, %[4]								
Tiabl et al., 2008	73	200	Estrus onset	96	2	59.4	72.4	0.5
Johnston et al., 2009[1]	300	.	24	96	1	72.7	76.7	0.43
Knox et al., 2011[2]	83	100	8, 32	96	2	78.4	65.6	0.39
Knox et al., 2011[2]	343	100	8, 32	96	2	56.4	56.3	0.36
Augsburger et al., 2012	202	200	24	96	1	83.3	81	0.74
Flowers et al., 2013[1]	398	.	20	96	1	76.7	78.5	0.46
Yeske et al., 2013	397	200	21-22	96	1	78.2	79.7	0.74
Total number of piglets born								
Knox et al., 2011[2]	83	100	8, 32	96	2	10	11.1	0.33
Knox et al., 2011[2]	343	100	8, 32	96	2	11.4	11.1	0.59
Johnston et al., 2013	205	200	24	96	1	13.5	13.4	0.82
Yeske et al., 2013	397	200	21-22	96	1	12.7	12.2	0.22
Number of piglets born alive								
Tiabl et al., 2008	73	200	Estrus onset	96	2	11.5	10.7	0.33
Johnston et al., 2009[1]	300	.	24	96	1	10.9	11.3	0.37
Knox et al., 2011[2]	83	100	8, 32	96	2	8.7	9.2	0.91
Knox et al., 2011[2]	343	100	8, 32	96	2	10.5	10.3	0.52
Augsburger et al., 2012	202	200	24	96	1	11.1	10.8	0.82
Flowers et al., 2013[1]	398	.	20	96	1	11.4	11.2	0.65
Yeske et al., 2013	397	200	21-22	96	1	11.8	11.3	0.17
Piglet index[5,6]								
Knox et al., 2011[2]	83	100	8, 32	96	2	682	604	.
Knox et al., 2011[2]	343	100	8, 32	96	2	604	579	.
Johnston et al., 2013	205	200	24	96	1	1094	1130	.

[a,b]Means within a row with different superscripts differ ($P < 0.05$).

[1]Amount of OvuGel® administered was not reported.

[2]Results of two trials reported in the same publication.

[3]Defined as the number of sows pregnant /number of sows allotted to OvuGel® or Control treatments.

[4]Defined as the number of sows farrowing/number of sows allotted to OvuGel® or Control treatments.

[5]Defined as the number of pigs born/number of sows allotted to OvuGel® or Control treatments.

[6]No statistics were reported for these measures.

Table 1.5. Summary of studies evaluating the effect of Matrix® and OvuGel® on day of expression of estrus based on day of first expression of estrus in gilts (Johnston et al., unpublished data).[1,2]

		Day of first expression of estrus[3]							
Item	# Gilts	5	6	7	8	9	10	Total Expressing Estrus	No estrus expression
Percentage of gilts first expressing estrus									
Johnston et al., 2013a[4]									
Control	137	1.5	29.9	38.0	13.9	2.9	0.7	87.6	12.4
OvuGel®	134	-	41.0	13.4	3.0	1.5	-	59.0	41.0
Johnston et al., 2013b[4]									
Control	134	5.2	60.4	25.4	3.0	-	2.2	97.2	3.0
OvuGel®	128	2.3	65.6	15.6	1.6	-	-	85.2	14.8
Johnston et al., 2013c[5]									
Control	148	2.7	28.4	24.3	13.5	6.1	1.4	81.8	18.2
OvuGel®	148	2.7	35.8	14.2	7.4	10.8	6.1	77.9	21.6

[1]Gilts administered Matrix® for 14 days. Gilts in OvuGel® treatment were administered OvuGel® 120 hours after last feeding of Matrix® and inseminated one time 24 hours later. Control gilts were inseminated as they were detected in estrus and inseminated every day that they remained in
[2]Studies presented are not published and no statistical analysis was performed on the data presented.
[3]Day 0 = day of last feeding of Matrix®.
[4]One gilt expressed estrus on day 13 which is not included in the table.
[5]Eight gilts displayed estrus on days 11-14 which are not included in the table.

Table 1.6. Summary of studies evaluating the effect of Matrix® and OvuGel® on the pregnancy and farrowing rate and litter size (Johnston et al., unpublished data).[1, 2]

	Treatment		
Item	Control	OvuGel® d 5	*P* - value
Number of gilts	148	148	
Pregnancy rate as a percentage of gilts allotted			
Johnston et al., 2013a	73.0[b]	83.1[a]	0.02
Johnston et al., 2013b	81.6	76.7	0.60
Johnston et al., 2013c	91.5[a]	82.5[b]	0.01
Farrowing rate as a percentage of gilts allotted			
Johnston et al., 2013a	71.6	77.5	0.17
Johnston et al., 2013b	75.0	67.9	0.38
Johnston et al., 2013c	89.3[a]	78.6[b]	0.02
Total number of piglets born per litter			
Johnston et al., 2013a	15.6[a]	13.2[b]	0.01
Johnston et al., 2013b	15.0	13.9	0.07
Johnston et al., 2013c	14.5	14.2	0.71
Number of piglets born alive per litter			
Johnston et al., 2013a	14.6[a]	12.5[b]	0.01
Johnston et al., 2013b	13.5	13.0	0.16
Johnston et al., 2013c	13.0	12.8	0.62

[a,b]Means within a row with different superscripts differ ($P < 0.05$).

[1]Gilts administered Matrix® for 14 days. Gilts in OvuGel® treatment were administered OvuGel® 120 hours after last feeding of Matrix® and inseminated one time 24 hours later. Control gilts were inseminated as they were detected in estrus and inseminated every day that they remained in estrus.

[2]Only control gilts that expressed estrus and were inseminated within 8 d after the end of Matrix® administration were included in the analysis.

CHAPTER 2. EFFECT OF TIMING OF OVUGEL ADMINISTRATION ON REPRODUCTIVE PERFORMANCE IN GILTS SYNCHRONIZED FOR ESTRUS.

ABSTRACT

The effect of time of OvuGel® administration on timing of estrus and ovulation and fertility results was evaluated in a study involving 448 gilts that had been synchronized for estrus by administering Matrix® for 14 d. A RCBD with the following treatments was used: 1) Control (no OvuGel® administration), 2) OvuGel® administration on d 5 (118 h) after the end of Matrix® administration (OV5), and 3) OvuGel® administration on d 6 (142 h) after the end of Matrix® administration (OV6). Control gilts were inseminated once per each morning of behavioral estrus; gilts administered OvuGel® received 1 insemination 24 h after OvuGel® administration. Gilts were randomly allotted to treatment within genetic line on the basis of similar live weight and stage of estrous cycle. Gilts were checked for estrus and ultrasonically scanned (trans-rectally; subsample of 250) at 12 h intervals from d 4 to 10 after Matrix® administration. Gilts on the OV5 and OV6 treatments exhibited estrus earlier ($P \leq 0.05$) than Control gilts (140.0, 140.9, and 151.3 h after the end of Matrix® administration, respectively; SEM 2.51). Ovulation occurred earlier ($P \leq 0.05$) for OV5 (164.8 h) than OV6 gilts (174.3 h) and earlier ($P \leq 0.05$) for OV6 than Control gilts (189.7 h; SEM 2.17). A greater ($P \leq 0.05$) percentage of Control gilts (100.0%) exhibited estrus at insemination than OV5 and OV6 gilts and more ($P \leq 0.05$) OV6 gilts (64.6%) exhibited estrus at insemination compared to OV5 gilts (40.4%). Gilts on the OV6 treatment had a lower ($P \leq 0.05$) interval from insemination to ovulation than OV5 gilts (8.2 vs. 22.8 h, respectively), and a greater ($P \leq 0.05$) percentage of OV6 gilts ovulated within 48 h after OvuGel® administration than OV5 gilts (92.5 vs. 70.9%, respectively). Pregnancy rates (percentage of total gilts allotted to the study that were pregnant at 35 d) and farrowing rates

(percentage of total gilts allotted to the study that farrowed) were greater ($P \leq 0.05$) for Control and OV6 than OV5 gilts (74.5, 56.9, and 79.0% for pregnancy rate and 73.8, 55.5, and 78.3% for farrowing rate for Control, OV5, and OV6, respectively). Pregnancy rates (percentage of total gilts inseminated that were pregnant at 35 d) and farrowing rates (percentage of total gilts inseminated that farrowed) were greater ($P \leq 0.05$) for Control than OV5 and OV6 gilts and greater ($P \leq 0.05$) for OV6 than OV5 gilts (91.0, 56.9 and 79.0% for pregnancy rate and 90.2, 55.5, and 78.3% for farrowing rate for Control, OV5, and OV6, respectively). Control gilts had a greater ($P \leq 0.05$) total number of piglets born per litter compared to OV5 gilts, however, OV6 had a similar ($P > 0.05$) total number of piglets born per litter compared to both Control and OV5 gilts (13.2, 12.1, and 12.6 for Control, OV5, and OV6, respectively; SEM 0.31). Control gilts had a greater ($P \leq 0.05$) number of piglets born alive per litter compared to both OV6 and OV5 gilts and OV6 gilts had a greater ($P \leq 0.05$) number of piglets born alive per litter compared to OV5 gilts (12.5, 11.2, and 11.7 for Control, OV5, and OV6 gilts, respectively; SEM 0.22). These results suggest that in gilts the timing of estrus and ovulation and fertility results depend on the time of OvuGel® administration following estrus synchronization.

Keywords: Gilt, OvuGel®, Ovulation, Estrus, Fertility

INTRODUCTION

OvuGel® (JBS United, Sheridan, IN), a product approved for use in sows, has been developed to synchronize ovulation and, thus, allow for a single fixed-time insemination for each female. This product may be administered regardless of the expression of behavioral estrus (according to the product label) and has been shown to effectively synchronize ovulation in a group of sows and produce similar fertility results compared to untreated control sows. However, little is known of the effect of OvuGel® on the timing of ovulation or fertility results in gilts. OvuGel® use is a simpler strategy in sows due to the fact that sows weaned on the same day should, subsequently, all be at a similar stage of estrous cycle. However, in a typical commercial situation groups of gilts are in estrus and ovulating at different times, thus, adding complexity to the use of this product in gilts. Matrix® administration can be used to synchronize estrus in a group of gilts, thus, allowing for the subsequent administration of OvuGel® and a single fixed-time insemination.

According to the product label, ovulation should occur 40 to 48 hours after OvuGel® administration, however, this is only true if there are follicles on the ovary capable of responding to the luteinizing hormone (LH) surge induced by OvuGel® administration. Three unpublished studies (Johnston et al. 2013a, b, and c) evaluated the effect of OvuGel® administration in gilts on fertility results, however, little is known of the effect of OvuGel® administration on follicular growth and time of ovulation. It is impossible, therefore, to determine if OvuGel® was administered at the appropriate time after Matrix® administration in these studies to allow for ovulation to be induced in response to the induced LH surge. Therefore, the objective of the current study was to evaluate the effect of the timing of OvuGel® administration on the timing of

estrus and ovulation and fertility results in gilts synchronized for estrus using Matrix® administered for 14 d.

MATERIALS AND METHODS

The study was conducted at Bible 4 Farm near Louisville, IL, which is a standard commercial breed to wean facility of The Maschhoffs (Carlyle, IL). The experimental protocol for the study was approved by the University of Illinois Institutional Animal Care and Use Committee.

Experimental Design and Treatments

The study was conducted as a randomized complete block design. Prior to application of treatments, all gilts were synchronized for estrus by administering Matrix® for 14 d. Date of start of Matrix® administration (contemporary group) was used as the blocking factor. There were 3 treatments: 1) Control (no OvuGel® administration), 2) OvuGel® administration on d 5 (118 h) after the end of Matrix® administration (OV5), and 3) OvuGel® administration on d 6 (142 h) after the end of Matrix® administration (OV6). Gilts allotted to the Control treatments were inseminated according to standard farm protocol and gilts allotted to OV5 and OV6 received 1 fixed-time insemination 24 h after OvuGel® was administered. The study began on the first day of Matrix® administration and ended when farrowing was completed.

Animals and Allotment to Study

A total of 448 post-pubertal gilts that were from 3 crossbred lines, based on Landrace and Yorkshire ancestry, were used for the study. Only gilts which had been observed in behavioral estrus in the 21-d period prior the beginning of Matrix® administration were used. For allotment to the study, gilts of the same genetic line were formed into outcome groups of 3 of similar body weight and at a similar stage of estrous cycle. Gilts were randomly allotted from within outcome

group (replicate) to 1 of the 3 treatments. After allotment to treatment, gilts from a contemporary group were moved to the same area of the barn.

Animal Housing and Management Prior to Start of the Study

All gilts were born and reared at the production unit in which the study was carried out. From birth until approximately 170 d of age gilts were housed and managed according to standard unit protocols.

Finisher Period 1 (from approximately 170 to approximately 207 d of age): At approximately 170 d of age, groups of 200 to 240 gilts of a similar age were moved into pens of 50 to 60 animals. Pigs had ad libitum access to feed and water. Boar exposure was initiated at approximately 170 d of age. A mature boar was allowed in each pen for a minimum duration of 2 h per d; however, estrus detection and recording was not carried out.

Finisher Period 2 (from approximately 207 to approximately 228 d of age): Beginning at approximately 190 d of age, the heaviest (based on visual observation) 45 to 60 animals from within the larger group of similar age were moved to smaller pens where they were kept in groups of 12 to 15 animals. This process continued on a weekly basis until all gilts from the group of similar age had been moved. Consequently, the age of gilts at the start of the Finisher Period 2 varied from approximately 190 to 224 d of age. Gilts had ad libitum access to feed and water. Daily estrus detection was carried out using boar exposure and the back pressure test. A mature boar was allowed into the pen and remained there for a minimum of 15 min. The date at which behavioral estrus was first observed was recorded. After approximately 21 d, all gilts that had exhibited estrus were weighed and moved to gestation crates and were allowed a 2-d acclimation period prior to the beginning of Matrix® (Merck Animal Health, Summit, NJ) administration.

Animal Housing and Management (Study Period)

The study consisted of 3 phases: Phase 1 (From initiation of Matrix® administration to d 10 after the last day of Matrix® administration), Phase 2 (Gestation [from d 11 after the last day of Matrix® administration to moving to farrowing room at approximately d 110 of gestation]), and Phase 3 (Farrowing [from moving to farrowing room through completion of farrowing]).

Phase 1 (from initiation of Matrix® administration to d 10 after the last day of Matrix® administration): Gilts were housed in individual crates with each crate being equipped with an individual nipple-type water drinker and an open feed trough that was supplied with feed via a drop feeder. Matrix® was administered at 0900 each day beginning on d 2 after the gilts were moved to crates and continuing for a 14-d period. The product was administered at a dosage of 15 mg/pig/d directly into the mouth using an application gun. Gilts were observed during the administration process and if it was considered that they did not ingest the full dose they were given another partial or full dose as needed. Estrus detection was carried out once per day (at approximately 0800) from the first day after the gilts were moved to gestation crates through 3 d after Matrix® administration was finished.

Phase 2 (Gestation [from d 11 after the last day of Matrix® administration to moving to farrowing room]): Approximately 5 d after insemination, gilts were moved to a gestation barn where they were housed in an individual crate until approximately d 110 of gestation. Each crate was equipped with an individual nipple-type water drinker and an open feed trough that was supplied with feed via a drop feeder.

In Phases 1 and 2, the ambient room temperature was maintained at approximately 20˚C using thermostatically controlled heaters and fan ventilation. Under hot conditions when the ambient room temperature reached 24.4˚ C water sprinklers were used in an attempt to cool pigs.

Phase 3 (Farrowing [from moving to farrowing room through completion of farrowing]): On approximately d 110 of gestation, gilts were moved from the gestation barn to a farrowing room where they remained until weaning. Each crate was equipped with an individual nipple-type water drinker and an open feed trough that was supplied with feed via a drop feeder. If a gilt had not begun to exhibit signs of farrowing by d 115 of gestation, they were induced to farrow by administering 1 dose of Lutalyse (2 mL, Zoetis, Florham Park, NJ). Ambient temperature within each farrowing room was maintained at 74° F during the time of farrowing.

Diet Formulation and Feeding

All diets fed throughout the study period were formulated to meet or exceed the nutrient requirements for breeding and gestating gilts recommended by NRC (2012). Gilts in Phase 1 and 2 of the study were fed twice daily (amount adjusted according to body condition). In Phase 1 they were fed at approximately 0600 and 0930 (with the second feeding being given immediately after Matrix® administration was completed). In Phase 2 they were fed at approximately 0600 and 1400). In Phase 3, gilts were fed twice daily (at approximately 0600 and 1400), however, if they were induced to farrow on the morning of d 115 of gestation, gilts were only fed in the morning (0600) and not fed again that day.

Estrus detection

Estrus detection was carried out using boar exposure and the back pressure test at 12 h intervals (0630 and 1830) beginning on the morning of d 4 after the last dose of Matrix® was administered and continuing until all gilts in the contemporary group had completed estrus (or until d 11 after the last d of Matrix® administration). A boar was allowed into the alleyway directly in front of the gilts and each gilt received a minimum of 2 min of direct boar exposure (defined as the ability to have physical nose to nose contact). During direct boar exposure, each

gilt was evaluated for the presence of behavioral estrus (standing reflex exhibited upon application of back pressure) and this was recorded accordingly. All measurements of times related to estrus were measured in hours after the last dose of Matrix® was administered. The start of estrus was defined as the first time behavioral estrus was exhibited minus 6 h. The end of estrus was defined as the first time behavioral estrus was not exhibited minus 6 h. The duration of estrus was measured as: (First time behavioral estrus was exhibited minus 6 h) to (First time behavioral estrus was not exhibited minus 6 h).

Trans-rectal ultrasounding

Trans-rectal real-time ultrasound scanning (SonoScape S8, Shenzhen, China; 5.0 MHz probe) was carried out on a subsample of approximately 65 gilts per treatment. Within each contemporary group, 5 to 8 replicates were chosen such that the mean weight and variation in weight and mean stage of estrous cycle and variation in stage of estrous cycle was similar to that of the contemporary group. Scanning was performed at 12 h intervals (at 0430 and 1630) beginning on the morning of d 4 after the last dose of Matrix® was administered and continuing until ovulation was confirmed. The number and size of ovarian follicles and time of ovulation was measured on one ovary of each gilt (the right ovary was used for the majority of measurements unless it could not be visualized during scanning, in which case the left ovary was used). Follicular size was calculated as the mean of the diameter of the three largest unovulated, non-cystic follicles (defined as follicles measures < 12.9 mm in diameter). Ovulation was determined to be completed when there was a clear reduction in follicle numbers from the previous scanning period and when there were 3 or fewer follicles measuring ≥ 6.5 mm in diameter remaining on the ovary. The time of ovulation was measured as the time ovulation was

completed (3 or fewer follicles remaining on the ovary) minus 6 h and was measured in hours after the last dose of Matrix® was administered.

OvuGel® Administration

OvuGel® was stored at approximately 5° C and the bottle was warmed to room temperature for a period of at least 10 min prior to administration. The product was administered intra-vaginally via a customized applicator. The infusion tube was covered with a protective sheath prior to insertion into the vagina and a new protective sheath was used for each gilt. Two ml of OvuGel® were administered to each gilt approximately 2 cm posterior to the cervix. OvuGel® was administered at 0700 on d 5 and d 6 after the last dose of Matrix® was administered to gilts on the OV5 and OV6 treatments, respectively, and regardless of whether the gilts exhibited behavioral estrus.

Insemination

All inseminations were carried out in the presence of a boar (similar to the process of estrus detection described previously). Each semen dose that was used contained 2.0×10^9 sperm cells in 80 ml of extended semen. Insemination occurred according to treatment.

Control treatment: Gilts on the Control treatment were inseminated according to standard farm protocol, which involved 1 insemination each morning (approximately 0800) that a gilt was determined to be in behavioral estrus. Estrus detection occurred twice daily for the purpose of the study, therefore, gilts on the Control treatment detected to be in estrus during the evening (1830) estrus detection were not inseminated at that time.

OvuGel® treatments: Gilts on the OvuGel® treatments received 1 insemination 24 h (0700) after OvuGel® administration and regardless of whether or not behavioral estrus was

exhibited. At the time of insemination, each gilt was observed for the presence of behavioral estrus and this information was recorded.

Gestation

Pregnancy detection was carried out via checking for estrus using a boar and the back pressure test (as described above) from d 11 to 35 after insemination and via trans-abdominal ultrasound scanning by a trained farm technician between d 28 and 35 after insemination (according to standard farm protocol). The animal identification and date of behavioral estrus or ultrasound confirmation of "not pregnant" were recorded.

Two methods were used to calculate pregnancy rate:

$$\frac{\text{Number of gilts confirmed pregnant by d 35 after insemination}}{\text{Number of gilts inseminated}} \times 100$$

$$\frac{\text{Number of gilts confirmed pregnant by d 35 after insemination}}{\text{Number of gilts allotted}} \times 100$$

Additionally, pregnancy rate was calculated for gilts that either showed or did not show behavioral estrus at insemination as follows:

$$\frac{\text{Number of gilts confirmed pregnant by d 35 after insemination that exhibited estrus at insemination}}{\text{Number of gilts that exhibited estrus at insemination}} \times 100$$

$$\frac{\text{Number of gilts confirmed pregnant by d 35 after insemination that did not exhibit estrus at insemination}}{\text{Number of gilts that did not exhibit estrus at insemination}} \times 100$$

Farrowing

After farrowing was completed, farm technicians collected and recorded the date of farrowing, total number of piglets born, born alive, born dead, and mummified), after which all litter data was validated by research personnel for accuracy.

The gestation length was calculated as the number of days from insemination to completion of farrowing. Two methods were used to calculate farrowing rate:

$$\frac{\text{Number of gilts farrowing}}{\text{Number of gilts inseminated}} \times 100$$

$$\frac{\text{Number of gilts farrowing}}{\text{Number of gilts allotted to the study}} \times 100$$

Additionally, farrowing rate was calculated for gilts that either showed or did not show behavioral estrus at insemination as follows:

$$\frac{\text{Number of gilts farrowing that exhibited estrus at insemination}}{\text{Number of gilts that exhibited estrus at insemination}} \times 100$$

$$\frac{\text{Number of gilts farrowing that did not exhibit estrus at insemination}}{\text{Number of gilts that did not exhibit estrus at insemination}} \times 100$$

Statistical Analysis

The PROC UNIVARIATE procedure of SAS (SAS Inst. Inc., Cary, NC) was used to verify normality and homogeneity of variances of the variables. Binary response variables were analyzed using the PROC FREQ procedures of SAS and the treatment effect and difference

between the means was analyzed using the chi-square test. All data that conformed to normality assumptions were analyzed using the PROC MIXED procedures of SAS (Littell et al., 1996). Data not conforming to normality assumptions and homogeneity of variances were transformed using the PROC RANK procedures of SAS. Individual gilt was the experimental unit for all measurements, and the model used accounted for the fixed effect of treatment and the random effect of replicate. Ovarian follicle number and size data were analyzed using the repeated measures option of PROC MIXED and the model used included the main effects of treatment and day of observation. Least-squares means for the Control, OV5, and OV6 treatments were separated using the PDIFF option of SAS with means being considered different at a $P \leq 0.05$.

RESULTS AND DISCUSSION

Gilt live weight and stage of estrous cycle prior to Matrix® administration

Least-squares means for the effect of Insemination Program on gilt live weight and stage of estrous at the start of the study (i.e, 2 d prior to and at the start of Matrix® administration, respectively) are presented in Table 2.1. There was no treatment effect ($P \leq 0.05$) on gilt live weight 2 d prior to the beginning of Matrix® administration or on the day of estrous cycle at the start of Matrix® administration, which was to be expected given that gilts were allotted to treatment on the basis of similar live weight and similar stage of estrous cycle.

Timing of estrus after Matrix® administration

Results for the timing of estrus are described in Table 2.1 where they are presented both as a percentage of the population exhibiting behavioral estrus on each day of estrus detection and as the least-squares means for the timing of the start and end of estrus and estrus duration, quantified in hours after the end of Matrix® administration. Estrus detection was carried out

from the day following the end of Matrix® administration; however, no gilts exhibited estrus until day 4 and, therefore, only the data from this day to the end of the estrus detection period will be presented and discussed.

Overall, more gilts on the Control treatment ($P \leq 0.05$) exhibited behavioral estrus between d 4 and 10 after Matrix® administration than gilts on the OvuGel® administration treatments. In addition, more gilts ($P \leq 0.05$) on the OV6 treatment compared to the OV5 treatment exhibited behavioral estrus between d 4 and 10 after Matrix® administration (Table 2.1). These results show that administering OvuGel® reduced the percentage of gilts that exhibited behavioral estrus during the estrus detection period, particularly for those animals administered the product on d 5 after the end of the Matrix® administration period.

The percentage of gilts expressing estrus in the estrus detection period for each day and also cumulatively over the period is presented in Table 2.1. Interestingly, there were no differences ($P > 0.05$) between the treatments for the percentage of gilts exhibiting estrus in the first 3 d (d 4, 5, and 6) of the estrus detection period (Table 2.1). However, the percentage of gilts exhibiting estrus was lower ($P \leq 0.05$) for those on the OV5 (3.4%) treatment than the other 2 treatments on d 7 (20.8 and 20.1%, for Control and OV6, respectively) and was lower ($P \leq 0.05$) for both the OV5 (2.1%) and OV6 (0.7%) treatments than the Control (8.7%) on d 8. There was no difference ($P > 0.05$) between the treatments for the percentage of gilts exhibiting estrus on d 9 and 10, which were the last 2 days of the detection period.

Similar to within day, there was no difference ($P > 0.05$) between treatments for the cumulative percentage of gilts exhibiting estrus from d 4 to 6 of the estrus detection period (Table 2.1). A greater ($P \leq 0.05$) cumulative percentage of both Control (67.8 and 76.5%, respectively) and OV6 gilts (70.8 and 71.5%, respectively) exhibited estrus on d 7 and 8 during

the estrus detection period, however, compared to OV5 gilts (56.9 and 58.9%, respectively). By d 9, a greater ($P \leq 0.05$) cumulative percentage of Control gilts (79.9%) exhibited estrus compared to OV5 (61.6%), however, a similar ($P > 0.05$) percentage of OV6 gilts (71.5%) exhibited estrus on d 9 compared to the other 2 treatments. By d 10 of the estrus detection period, a greater ($P \leq 0.05$) percentage of Control gilts (81.9%) had exhibited estrus compared to both OV5 (61.6%) and OV6 (72.2%). These results indicate that OvuGel® administration decreased the percentage of gilts exhibiting behavioral estrus, and this decrease was primarily observed from d 2 after OvuGel® administration for both OV5 and OV6 gilts (i.e., d 7 and 8 of the estrus detection period for the OV5 and OV6 treatments, respectively). Interestingly, only 8.2% of all OV5 gilts expressed estrus after d 6 and only 1.4% of all OV6 gilts expressed estrus after d 7 after the end Matrix® administration; whereas 34.7% and 14.1% of Control gilts expressed estrus after d 6 and 7 after the end of Matrix® administration, respectively.

There are currently no published studies evaluating the effect of OvuGel® administration on the timing of estrus in gilts. Johnston et al. (2013a, b, and c; Table 1.6) conducted 3 studies evaluating the effect of OvuGel® administration on d 5 after Matrix® administration on timing of estrus, and the results of these studies will be compared and contrasted with the results of the current study (for the Control and OV5 treatment). It should be noted, however, that no statistical analysis was completed on the studies of Johnston et al. (2013a, b, and c).

The results from the present study relating to the percentage of gilts first displaying estrus within each day after Matrix® administration are generally in line with those reported by Johnston et al. (2013a, b, c). On d 5 after Matrix® administration, however, a greater percentage (mean of approximately 19% between treatments) of Control and OV5 gilts in the current study displayed estrus than reported by Johnston et al. (2013a, b, and c; mean of approximately 3.0%

between treatments in all studies; Table 1.6). When comparing the overall percentage of gilts displaying estrus after Matrix® administration, the results of the current study were generally in agreement with those of Johnston et al. (2013a and c), however, Johnston et al. (2013b) reported a larger percentage (approximately 12.5 percentage units higher) of gilts displaying estrus after Matrix® administration compared to the current study. It is important to note, however, when comparing these studies, that Johnston et al. (2013a, b, and c) evaluated gilts for behavioral estrus through d 14 following Matrix® administration, whereas in the current study gilts were only observed through d 10 after Matrix® administration, therefore, a larger reported percentage of gilts displaying estrus in Johnston et al. (2013a, b, and c) might be expected.

Although, as previously mentioned, more gilts in the current study expressed estrus on d 5 after the end of Matrix® administration than was reported by Johnston et al. (2013a, b, and c), in all 4 studies, the majority of Control gilts and gilts administered OvuGel® on d 5 after Matrix® administration first displayed estrus between d 5 and 7 after the end Matrix® administration (range of 52.7 to 91.0% of gilts across studies). Additionally, for gilts administered OvuGel® on d 5 after Matrix® administration, a decrease in the percentage of gilts displaying estrus from d 6 and 7 occurred in all 4 studies (range of 21.6 to 50.0 percentage unit decrease across studies). This decrease in estrus occurrence on d 7 would appear to be consistent with when ovulation would have been expected to have occurred after OvuGel® administration on d 5 which according to JBS United (2014) would be between 40 to 48 h after administration. Gilts administered OvuGel® on d 6 in the current study also appeared to have followed a similar pattern in relation to the numbers exhibiting estrus which decreased by 19.4 percentage units from d 7 to 8; based on the information available relating to OvuGel® treatment at d 5 after Matrix®, ovulation would have been expected to occur prior to d 8, however, there is no other

available data to compare these results with. When evaluating the success of OvuGel® administration solely from the timing of behavioral estrus, these results seem to indicate that OvuGel® administration at either d 5 or 6 after Matrix® administration were equally successful with the majority of gilts appearing to have ovulated within the necessary time frame described above (within 48 h after administration). JBS United (2014), however, reports that behavioral estrus may not be necessary in order for ovulation within the necessary time frame to occur. The results for the timing of ovulation and pregnancy rates, therefore, need to be evaluated to fully understand the effect of OvuGel® administration at different times and its effect on both the timing of ovulation and subsequent fertility.

It is important to also compare the direct effects of Matrix® administration and its effect on the synchronization of estrus in the current study (Control treatment; not administered OvuGel®) with the results of other studies; however, this is difficult because there is little published literature that has used the same dosage and duration of Matrix® as the current study. Johnston et al. (2013a, b, and c [unpublished]) and Rhodes et al. (1991) each used a similar dosage and duration of Matrix® administration to those used in the current study and those results will be compared with those of the Control treatment from the current study. Johnston et al. (2013c) reported that a higher percentage (14.3 percentage units) of gilts displayed estrus by d 10 after Matrix® administration than found in the current study. Rhodes et al. (1991) and Johnston et al. (2013a and b), however, reported a higher percentage (5.0 to 8.1 percentage) of gilts displaying estrus by d 10 after Matrix® administration compared to the current study. Although 3 of the 4 studies reported relatively similar results compared to those of the current study, there was considerable variation in the percentage of gilts displaying estrus after Matrix® administration across studies. This is particularly important because it has previously been

suggested that the response of gilts to Matrix® synchronization may be highly variable between different populations of gilts, with factors such as genetics, environment, and management having an effect on the synchronization of both estrus and ovulation. If different populations of pigs respond differently to Matrix® administration, implementing OvuGel® into a breeding program could also result in differing levels of success.

Least–squares means for the effect of timing of OvuGel® administration on the start, end and duration of estrus are presented in Table 2.1. Gilts administered OvuGel® for OV5 and OV6, respectively) exhibited behavioral estrus earlier ($P \leq 0.05$) after the end of Matrix® administration than Control gilts (140.0, 140.9, and 151.3 h for OV5, OV6, and Control, respectively). In contrast, Control gilts exhibited estrus for a longer duration ($P \leq 0.05$) than OV5 however, OV6 had a similar ($P > 0.05$) estrus duration compared to both the Control and OV5 gilts (49.1, 41.1, 47.3 h for Control, OV5 and OV6 gilts, respectively). In addition, the end of estrus occurred later ($P \leq 0.05$) in Control gilts than in gilts administered OvuGel® (196.7, 179.3 and 188.2 h after the end of Matrix® for Control, OV5 and OV6 gilts, respectively), and later ($P \leq 0.05$) after the end of Matrix® administration in OV6 than OV5 gilts. These results indicate that OvuGel® administration decreased the time to estrus onset and that estrus duration was shortest in gilts administered OvuGel® on d 5 after Matrix® administration. The results for the start of estrus and end of estrus also seem to agree with the results for the percentage of gilts exhibiting estrus within each day as described above, as a relatively small percentage of OV5 and OV6 gilts exhibited estrus subsequent to 48 h after OvuGel® administration, whereas the expression of estrus was more widely distributed between d 4 to 10 after Matrix® administration for Control gilts.

Least-squares means for the timing of first occurrence of estrus for the Control gilts from the current study was generally in line with previous studies that measured the timing of first occurrence of estrus after 14 d of Matrix® administration (Control treatment; Davis et al. 1987a, Davis et al. 1987b, and Rhodes et al. 1991). There are no published reports on the effects of Matrix® administration in gilts on the duration of estrus, however, the results from the current study are generally in line with previous research evaluating estrus duration in gilts that were not synchronized for estrus using Matrix® (Eliason, 1989, Steverink et al., 1999, Almeida et al., 2000, Bortolozzo et al., 2005, Horsley et al., 2005).

Least-squares means for the effect of timing of OvuGel® administration on the percentage of gilts exhibiting estrus at the time of insemination are presented in Table 2.1. A greater percentage of Control gilts ($P \leq 0.05$) exhibited estrus at insemination compared to OV5 and OV6 and more OV6 gilts ($P \leq 0.05$) exhibited behavioral estrus at insemination than OV5 gilts (100.0, 40.4, and 64.6% for Control, OV5, and OV6 gilts, respectively). It is important to note that Control gilts were only inseminated if they exhibited behavioral estrus, whereas, gilts administered OvuGel® were inseminated regardless of whether they exhibited behavioral estrus. These results suggest that OvuGel® administration at both times influenced the incidence of behavioral estrus at insemination. It could be hypothesized that OvuGel® administration on d 6 may have occurred more synchronously with the "normal" timing of estrus than OvuGel® on d 5 after Matrix® administration.

Least-squares means for the effect of timing of OvuGel® administration on number of inseminations per gilt are presented in Table 2.1. As expected, Control gilts received a greater ($P \leq 0.05$) number of inseminations compared to gilts administered OvuGel®. Gilts administered OvuGel® received only 1 insemination at a fixed time (24 h after OvuGel® administration),

however, Control gilts received 1 insemination for each morning they exhibited behavioral estrus.

In conclusion, evaluating the effectiveness of Matrix® administration on the synchronization of estrus in a group of gilts is important in studies that combine Matrix® and OvuGel® administration. OvuGel® administration will not cause ovulation unless the gilt has follicles that are mature enough to respond to the induced LH surge; consequently, using Matrix® to effectively synchronize estrus in an entire group of gilts is necessary for the practical use of OvuGel® in gilts. In this study and in previously unpublished research (Johnston et al., 2013a, b, and c), it appears that OvuGel® administration has an effect on the timing and incidence of estrus after Matrix® administration, however, further research is necessary to understand this relationship. Finally, it is important to note that aside from the current study, there is no research that has evaluated the effect of OvuGel® administration to gilts on d 6 after Matrix® administration. Results from the current study suggest that OvuGel® administration on d 6 after Matrix® administration may result in a greater percentage of gilts displaying estrus at insemination and a greater percentage of gilts having displayed estrus by d 10 after Matrix® administration, however, further research is necessary to validate and further understand these findings.

Timing of ovulation

A subsample of gilts (n = ~250) were ultrasonically scanned (trans-rectally) at 12 h intervals from d 4 to 10 after Matrix® administration to determine the timing of ovulation. The results for the effect of Insemination Program on the percentage of gilts exhibiting estrus, least-squares means for the time interval from the end of Matrix® administration to the start and end of estrus, estrus duration, time interval from start of estrus to ovulation, timing of ovulation as a

percentage of estrus duration, and follicle number and size prior to ovulation are presented in Table 2.2. The least-squares means for the time interval from the end of Matrix® administration to the start and end of estrus and the duration of estrus (Table 2.2), were generally in line with the same data reported for the entire population of animals (Table 2.1); therefore these results will only be discussed in the context of how they are related to the results describing the timing of ovulation. The results for the effect of Insemination Program on the timing of ovulation for each day and cumulatively for d 4 to 10 after the end of Matrix® administration are presented in Table 2.3, and the least-squares means for the effect of timing of OvuGel® administration on the time interval from the end of Matrix® administration to ovulation, OvuGel® administration to ovulation, insemination to ovulation, and the percentage of gilts ovulating after OvuGel® administration and insemination are presented in Table 2.4 and illustrated in Figures 2.1, 2.2, and 2.3.

There are no published studies evaluating the effect of OvuGel® administration on the timing of ovulation in gilts. The results for timing of ovulation, however, will be compared and contrasted with studies that have evaluated the timing of ovulation in gilts that were not administered OvuGel®, and the timing of ovulation in sows administered OvuGel®.

Timing of ovulation after start of estrus and timing of ovulation as a percentage of estrus duration Least-squares means for the time interval to ovulation after the start of estrus and the timing of ovulation as a percentage of estrus duration are described in Table 2.2. Control and OV6 gilts had a greater ($P \leq 0.05$) interval from the start of estrus to ovulation than OV5 gilts (34.2, 34.0, and 26.0 h, for Control, OV6, and OV5 gilts, respectively). There was also a trend ($P = 0.06$) for gilts administered OvuGel® to have a greater ovulation time as a percentage of estrus duration compared to Control gilts (74.3, 76.4, and 68.6% for OV5, OV6, and Control

gilts, respectively), however, the treatment differences were relatively small. These results suggest that OV5 gilts had the shortest interval to ovulation after the start of estrus, which would appear to be in agreement with the duration of estrus being shorter ($P \leq 0.05$) for OV5 gilts compared to Control and OV6 gilts.

Results for the time interval from the start of estrus to ovulation are generally in line with previous research that has evaluated this measure in gilts not administered Matrix® or OvuGel® (Bracken et al., 2003 and Bortolozzo et al., 2005). Almeida et al. (2000) reported a greater time interval from the start of estrus to ovulation (43.9 h) in gilts than what was found in the current study, however, a longer estrus duration was also reported in the study of Almeida et al. (2000), therefore, a higher time interval to ovulation may be expected. Additionally, Knox et al. (2011) reported that sows administered OvuGel® 96 h after weaning and inseminated 24 h later had an average time interval to ovulation after the start of estrus of 34.6 h, which is also generally in line with the results from the current study.

Compared to the results of the current study, Almeida et al. (2000) and Bracken et al. (2003) reported higher (85.7%) and lower (59.5%) means, respectively, for time of ovulation as a percentage of estrus duration in gilts not administered Matrix® or OvuGel®. Several other studies conducted in both gilts and sows, however, have reported values for the time of ovulation as a percentage of estrus duration that were generally in line with the results of the current study (range of 68 to 72% across studies; Soede et al., 1995; Nissen et al., 1997; Steverink et al., 1997 and Bortolozzo et al., 2005). In addition, Soede and Kemp (1997) reported that on average, ovulation occurs 70% of the way through the estrus duration and the results of the current study would generally agree with this statement.

Follicle number and size The results for ovarian follicle number and mean follicle size 12 h prior to ovulation are reported in Table 2.2. One ovary from each gilt was used in the measurement of both variables and the mean of the measurement taken on the 3 largest follicles was used in the determination of mean follicle size. There was no difference ($P > 0.05$) between treatments for the number of follicles present at 12 h prior to ovulation, however, Control and OV6 gilts had a larger ($P \leq 0.05$) mean follicle size compared to OV5 at 12 h prior to ovulation (6.6, 6.4, and 6.1 mm for Control, OV6, and OV5 gilts, respectively). These results indicate that at the time of ovulation, gilts administered OvuGel® on d 5 had smaller and, in theory, less mature follicles compared to the other 2 treatments, and this may have an impact on subsequent fertility.

Percentage ovulating within day and cumulatively within day after Matrix® administration The results for the total percentage of gilts ovulating between d 4 and 10 after Matrix® administration are presented in Table 2.3. Overall, there was no treatment difference ($P > 0.05$) for the percentage of gilts ovulating between d 4 and 10 after Matrix® administration, however, there was a trend ($P = 0.10$) for a higher percentage of OV6 gilts to have ovulated within this period (95.7, 88.1, and 84.8% for OV6, Control, and OV5 gilts, respectively). These results suggest that administering OvuGel® at either 5 of 6 d after the end of Matrix® administration had no statistically significant impact of the percentage of gilts ovulating by d 10 after Matrix® administration. In the current study, however, the time interval to ovulation in relation to the timing of Matrix® and OvuGel® administration and insemination is of greater relevance and will be discussed later.

The percentage of gilts ovulating within each day after Matrix® administration and cumulatively each day after Matrix® administration are presented in Table 2.3. Gilts were

ultrasonically scanned at 12 h intervals from d 4 to 10 after Matrix® administration until ovulation was confirmed; however, the results that will be described from Table 2.3 are presented as the total percentage that ovulated each day (the percentage of gilts ovulating at each 12 h interval are described in Appendix Table 2.4). There were no differences ($P > 0.05$) between the treatments for the percentage of gilts ovulating in the first 3 d (d 4, 5, and 6) of the scanning period and it is important to note that a small percentage of gilts (between 2.5 and 6.0% depending on treatment) ovulated during this time period. However, the percentage of gilts ovulating on d 7 after Matrix® administration was higher ($P \leq 0.05$) for OV5 gilts than the other 2 treatments and was higher ($P \leq 0.05$) for OV6 than Control gilts (22.4, 74.7, and 37.7% for Control, OV5, and OV6 gilts, respectively). In contrast, on d 8 after Matrix® administration, more ($P \leq 0.05$) Control and OV6 gilts ovulated compared to OV5 gilts (37.3, 3.8, and 52.2% for Control, OV5, and OV6 gilts, respectively) and more ($P \leq 0.05$) Control gilts ovulated on d 9 after Matrix® administration than for the other 2 treatments (16.4, 1.3, and 0.0% for Control, OV5, and OV6 gilts, respectively). However, there were no treatment differences ($P > 0.05$) for the percentage of gilts ovulating on d 10 after Matrix® administration.

Cumulatively, there were no treatment differences ($P > 0.05$) for the percentage of gilts ovulating on d 4, 5, and 6 after Matrix® administration (Table 2.3). By d 7 after Matrix® administration, however, more ($P \leq 0.05$) OV5 gilts had ovulated compared to the other 2 treatments, and more ($P \leq 0.05$) OV6 gilts had ovulated than Control gilts (28.4, 77.2 and 43.5% for Control, OV5, and OV6, respectively), whereas by d 8, more ($P \leq 0.05$) OV6 gilts had ovulated than the other 2 treatments and more ($P \leq 0.05$) OV5 gilts had ovulated than Control gilts (65.7, 81.0, and 95.7% for Control, OV5, and OV6, respectively). Similarly, more ($P \leq 0.05$) OV6 gilts had ovulated by d 9 after Matrix® administration than the other 2 treatments

(82.1, 82.3, and 95.7% for Control, OV5, and OV6, respectively), however, by d 10 after Matrix® administration for the cumulative number of gilts ovulating was similar ($P < 0.05$) for the 3 treatments. These results demonstrate that OvuGel® administered at both time points (d 5 and 6 after Matrix® administration) caused ovulation to occur earlier in a substantial proportion of gilts. It is interesting to note, however, that the distribution in when ovulation occurred in the 2 d following OvuGel® administration differed between treatments, with a substantial proportion of OV5 gilts ovulating on the second day after OvuGel® administration (74.7% (versus 2.5% on the first day), whereas for OV6 the distribution between days was more spread out (37.7 and 52.2% ovulating on the first and second days after OvuGel® administration, respectively.

Time interval from end of Matrix® administration to ovulation Least-squares means for the effect of timing of OvuGel® administration on the time interval from the end of Matrix® administration to ovulation are described in Table 2.4 and Figure 2.1. Gilts administered OvuGel® on d 5 after Matrix® administration ovulated earlier ($P \leq 0.05$) than the other 2 treatments, and OV6 gilts ovulated sooner ($P \leq 0.05$) than Control gilts (164.8, 174.2, 189.7 h for OV5, OV6 and Control gilts, respectively). Figure 2.1 illustrates the frequency distribution of when gilts in each treatment ovulated after Matrix® administration. Interestingly, the peak for when the largest number of OV5 gilts ovulated occurred at 160 h after Matrix® administration, with relatively few gilts ovulating before or after. In contrast, however, although there was also a peak for when the greatest number of gilts ovulated, ovulation was more widely distributed across time points for Control and OV6 gilts. These results suggest that OvuGel® administration decreased the average time interval to ovulation after Matrix® administration and that the timing of OvuGel® administration also impacted the distribution for the time when gilts ovulated.

Time interval from OvuGel® administration to ovulation The results for the time interval from OvuGel® administration to ovulation are presented in Table 2.4 and illustrated in Figure 2.2. Gilts administered OvuGel® on d 5 after Matrix® administration had a greater ($P \leq 0.05$) time interval from OvuGel® administration to ovulation than gilts administered OvuGel® on d 6 (46.8 and 32.2 h, respectively). Interestingly, Figure 2.2 illustrates that although for both treatments, the highest peaks for when the largest number of gilts ovulated occurred at approximately the same time in relation to the timing of OvuGel® administration, a higher number of OV6 gilts ovulated prior to this peak; whereas the vast majority of all OV5 gilts ovulated at one time point (Figure 2.2). These results suggest that timing of OvuGel® administration had an effect on the timing and distribution of when ovulation occurred in gilts.

Time interval from insemination to ovulation The results for the time interval from insemination to ovulation for the 2 treatments administered OvuGel® are reported in Table 2.4 and illustrated in Figure 2.3. Gilts administered OvuGel® on d 5 after Matrix® administration had a greater ($P \leq 0.05$) time interval from insemination to ovulation than gilts administered OvuGel® on d 6 (22.8 and 8.2 h for OV5 and OV6 gilts, respectively). When comparing only gilts that ovulated after insemination; OV5 gilts still had a greater ($P \leq 0.05$) time interval from insemination to ovulation than OV6 gilts (22.8 and 14.5 h for OV5 and OV6 gilts, respectively). There were no gilts on the OV5 treatment that ovulated prior to insemination, however, for gilts on the OV6 treatment 15 out of 66 (22.7%) ovulated prior to insemination and the mean time interval from ovulation to insemination for these gilts was 13.2 h. The frequency distribution for the time interval from insemination to ovulation is also illustrated in Figure 2.3, and this shows that although the time when the largest number of gilts ovulated relative to insemination was

similar between treatments (as previously discussed), a greater number of OV6 compared to OV5 gilts ovulated prior to insemination.

Percentage ovulating within 48 h after OvuGel® administration Results for the percentage of gilts ovulating within 48 h after OvuGel® administration are presented in Table 2.4. A lower number ($P \leq 0.05$) of OV5 gilts ovulated within 48 h after OvuGel® administration than OV6 gilts (70.9 and 92.5% for OV5 and OV6, respectively). According to the product label, OvuGel® administration should cause ovulation within 40 to 48 h after administration (JBS United, 2014) and although the majority of gilts administered OvuGel® at both time points ovulated within this time frame, a higher percentage (21.6%) of OV6 gilts ovulated in this time frame compared to OV5 gilts.

Percentage ovulating within 24 h of insemination The results for the percentage of gilts administered OvuGel® ovulating within 24 h before ovulation are presented in Table 2.4. There was no difference ($P > 0.05$) in the percentage of gilts ovulating within 24 h prior to insemination (70.9 and 75.4% for OV5 and OV6 gilts, respectively). The time interval from 24 h prior to and 4 h after ovulation is cited by several authors as the time of insemination when the highest fertility can be achieved (Waberski et al., 1994; Nissen et al., 1997; Soede et al., 1995; Steverink et al., 1997; Bracken et al., 2003). The results of the current study suggest that for both timing of OvuGel® administration treatments, the majority of gilts ovulated within 24 h after insemination; however, 22.7% of OV6 gilts ovulated from 6 to 54 h prior to insemination (0.0% of OV5 gilts ovulated in that same period), which could have negative effects on fertility.

As previously mentioned, according to the product label, OvuGel® is expected to induce ovulation within 40 to 48 h after administration (JBS United, 2014), however, it could by hypothesized that a greater percentage of gilts on the OV6 treatment ovulated as a result of the

natural occurrence of ovulation following Matrix® administration, rather than ovulation having been induced by the OvuGel® administration. The following findings from the current study support this hypothesis:

- The time of ovulation was more widely distributed across time points for OV6 gilts compared to gilts on the OV5 treatment.
- While the vast majority of gilts on the OV5 treatment ovulated 40 to 48 h after OvuGel® administration, a proportion of OV6 gilts ovulated prior to this 40 to 48 h time period after OvuGel® administration.
- More gilts on the OV5 treatment did not ovulate within 48 h after OvuGel® administration compared to OV6 gilts (29.1 and 7.5% for OV5 and OV6 gilts, respectively).
- A proportion (22.7%) of gilts on the OV6 treatment ovulated after insemination (no gilts on the OV5 treatment ovulated after insemination).
- A greater ($P \leq 0.05$) percentage of OV6 gilts than OV5 gilts exhibited estrus at insemination (as described in Table 2.1).

As previously described, gilts on the OV5 treatment had a smaller mean ovarian follicle size prior to ovulation compared to OV6, and less OV5 gilts ovulated within 48 h of OvuGel® administration compared to gilts administered OvuGel® on d 6 after Matrix® administration. It could also be hypothesized, therefore, that some OV5 gilts may not have had ovarian follicles mature enough to respond to ovulation induction at the time of OvuGel® administration; thus, OvuGel® administration on d 5 was too early.

In conclusion, OvuGel® administration decreased the time interval to ovulation in gilts compared to the Control gilts, and OvuGel® administration at different time points had an impact on the time interval to ovulation. In addition, OvuGel® administered on both d 5 and 6 after Matrix® administration resulted in the majority of gilts ovulating within 40 to 48 h of OvuGel® administration. However, OvuGel® administration on d 6 following Matrix® administration seemed to occur more closely with the natural timing of ovulation following Matrix® administration and administration on d 5 may have been too early for ovulation to be induced. Before any conclusions can be drawn on the impact of Insemination Program (including OvuGel® administration at both time points), however, further research is necessary to understand the time interval to ovulation after Matrix® and OvuGel® administration and to understand the impact of Insemination Program on subsequent fertility results.

Pregnancy and farrowing rate, gestation length, and litter size

The effect of Insemination Program treatment on pregnancy and farrowing rates and litter size is presented in Table 2.5. All gilts allotted to receive OvuGel® were inseminated independent of whether they were in behavioral estrus or not, however, gilts allotted to the Control were only inseminated if they expressed behavioral estrus. Therefore, the pregnancy and farrowing rates were calculated 2 ways, firstly based on the number of gilts allotted to the study and secondly on the number of gilts inseminated. Pregnancy and farrowing rates for the OV5 and OV6 treatments were also calculated both for gilts that exhibited behavioral estrus and also those for that did not exhibit behavioral estrus at the time of insemination.

Pregnancy and farrowing rates The effect of Insemination Program treatment on pregnancy and farrowing rates are presented in Table 2.5. Control and OV6 gilts had a higher ($P \leq 0.05$) pregnancy and farrowing rate as a percentage of gilts allotted to the study compared to

OV5 gilts (74.5, 79.0, and 56.9% for pregnancy rate and 73.8, 78.3, and 55.5% for farrowing rate for Control, OV6, and OV5 gilts, respectively). Control gilts had a higher ($P \leq 0.05$) pregnancy and farrowing rate as a percentage of all gilts inseminated compared to OV6 and OV5 gilts, and OV6 gilts had a higher ($P \leq 0.05$) pregnancy and farrowing rate as a percentage of gilts inseminated compared to OV5 gilts (91.0, 79.0, and 56.9% for pregnancy rate and 90.2, 78.3, and 55.5% for farrowing rate for Control, OV6, and OV5 gilts, respectively). In total, 27 Control gilts did not exhibit behavioral estrus during the estrus detection period (d 4 to 10 after Matrix® administration), which accounts for the difference between the pregnancy and farrowing rates for this treatment when expressed as a percentage of gilts allotted to the study compared to the percentage of gilts actually inseminated.

These results show that when gilts on the Control treatment that did not exhibit behavioral estrus within 10 d after Matrix® administration (and were, therefore, not inseminated) were factored into the pregnancy and farrowing rate calculation, gilts that were administered OvuGel® on d 6 after Matrix® administration had similar pregnancy and farrowing rates compared to the Control treatment which was a traditional insemination program.

The pregnancy and farrowing rates calculated as a percentage of either gilts exhibiting estrus at insemination or gilts not exhibiting estrus as insemination are presented in Table 2.5. Control and OV6 gilts that were in estrus at the time of insemination had higher ($P \leq 0.05$) pregnancy and farrowing rates compared to OV5 gilts (91.0, 91.4, and 74.6% for pregnancy rate and 90.2, 91.4, and 74.6% for farrowing rate for Control, OV6, and OV5 gilts, respectively). For the pregnancy and farrowing rates calculated as a percentage of gilts that were not in estrus at insemination, Control gilts were not included in the analysis because they were only inseminated if they exhibited estrus (therefore, there were no Control gilts that did not exhibit estrus at

insemination). There was no difference ($P > 0.05$) between OV5 and OV6 gilts for the pregnancy and farrowing rates of gilts that were not in estrus at the time of insemination. These results suggest that when comparing only gilts that were in estrus at the time of insemination, less OV5 gilts were pregnant and farrowed after insemination compared to both Control and OV6 gilts. This may suggest that, as previously noted, OvuGel® administration on d 5 after Matrix® administration may be too early in regards to the degree of follicular maturity at the time of OvuGel® administration. In addition, even though gilts administered OvuGel® were inseminated regardless of estrus, only approximately half of the gilts from both treatments became pregnant and farrowed, which may indicate that despite ovulation induction with OvuGel®, the physical occurrence of estrus at the time of insemination is still an important precursor to conception.

In the unpublished studies by Johnston et al. (2013a, b, and c) the pregnancy and farrowing rates were only calculated as a percentage of gilts allotted to the study, and these are presented in Table 1.6. The gilts allotted to receive OvuGel® in these studies were all administered OvuGel® on d 5 after Matrix® administration, and, therefore, the results can only be compared with the results for the gilts on the OV5 treatment in the current study. One study reported a lower ($P \leq 0.05$) pregnancy and farrowing rate for gilts administered OvuGel® compared to the Control, which was in agreement with the current study. In contrast, another study reported a higher ($P \leq 0.05$) pregnancy rate and no difference ($P > 0.05$) in the farrowing rate between gilts administered OvuGel® and Control gilts, and the final study reported no difference ($P > 0.05$) between treatments for both pregnancy and farrowing rate. In general, in the current study OV5 gilts had numerically lower pregnancy and farrowing rates (~12 to 25% lower) than those reported for gilts administered OvuGel® on d 5 after Matrix® administration in

the studies of Johnston et al. (2013a, b, and c). It should be noted, however, that the increased pregnancy rates reported in Johnston et al. (2013a, b, and c) could be partly attributed to the different criterion used for stage of estrous cycle at the start of Matrix® administration compared to the current study. Gilts in the studies of Johnston et al. (2013a, b, and c) were only included if they were between d 4 and 16 of their estrous cycles (with d 0 equating to first day of estrus) at the start of Matrix® administration, whereas, in the current study gilts in all stages of the estrous cycle were included in the study. This would have resulted in a large proportion of these gilts having been at a stage of estrous cycle more synchronous with the natural occurrence of estrus 5 d after the end of Matrix® administration than in the current study, which could have had a positive impact on pregnancy rates.

Gestation length The least-squares means for the effect Insemination Program treatment on gestation length are presented in Table 2.5. For Control gilts, the gestation length was calculated using the first day of insemination (the majority of Control gilts were inseminated over more than one day). There was no difference ($P > 0.05$) between treatments for gestation length. However, care needs to be taken when interpreting these results because conception may not have occurred on the first day of insemination for Control gilts and, therefore, the calculation of gestation length for this treatment may not be entirely accurate.

Litter size The effect of Insemination Program treatment on litter size is presented in Table 2.5. Control gilts had a higher ($P \leq 0.05$) total number of piglets born per litter compared to OV5 gilts and a higher ($P \leq 0.05$) number of piglets born alive compared to both OV5 and OV6 gilts, however, there was no difference ($P > 0.05$) between OV6 and Control gilts and OV6 and OV5 gilts for total number of piglets born and no difference ($P > 0.05$) between OV5 and OV6 gilts for number of piglets born alive (13.2, 12.6, and 12.1 for total number of piglets born

and 12.5, 11.7, and 11.2 for number of piglets born alive for Control, OV6, and OV5 gilts, respectively). These results show that OvuGel® administration on d 5 after the end of Matrix® administration reduced both the total number of piglets born and born alive and OvuGel® administration on d 6 after Matrix® administration also reduced the number of piglets born alive compared to Control gilts.

These results may imply that OvuGel® administration on d 5 after the end of Matrix® administration may have been too early in regards to follicle maturity at the time of induced ovulation. Although there was no treatment difference for the number of follicles at 12 h prior to ovulation, OV5 gilts did have a smaller mean follicle size 12 h prior to ovulation (as described in Table 2.2), thus, the oocytes for OV5 gilts may have been less mature at the time of fertilization compared to the other treatments. That OvuGel® administration on d 5 after Matrix® administration may have been too early for maximum fertility is also supported by the overall poorer response to OvuGel® administration on d 5 compared to the Control and gilts administered OvuGel® on d 6 as shown in the results for the timing of ovulation (described in Table 2.5) and for pregnancy and farrowing rates.

In total, 95% of the Control gilts that were inseminated received more than 1 insemination, and it could be hypothesized that the multiple inseminations given over the duration of the estrus period may have contributed to the increased number of piglets born alive per litter for the Control versus OV6 gilts. Multiple inseminations throughout the duration of the estrus period could have resulted in a greater number of fertile sperm cells in the reproductive tract throughout the entire estrus period, thus potentially increasing the number of fertilized oocytes for Control gilts. It is difficult, however, to clearly determine the cause for the greater number of piglets born alive to Control gilts compared to OV6 gilts. In theory, a more

appropriate timing of OvuGel® administration or insemination could also have increased the number of piglets born alive and further research to determine the optimum time of OvuGel® administration and for insemination is warranted.

One of the unpublished studies by Johnston et al. (2013a, b, and c) reported that Control gilts had a higher ($P \leq 0.05$) total number of piglets born and born alive per litter compared to gilts administered OvuGel® on d 5 after the end of Matrix® administration, which is in agreement with the current study. The 2 other studies, however, reported that there was no difference ($P > 0.05$) between the Control treatment and gilts administered OvuGel® for total number of piglets born or born alive per litter, which would be in disagreement with the current study. It should be noted, however, that there was a trend ($P = 0.07$) for Control gilts in Johnston et al. (2013b) to have a higher total number of piglets born compared to gilts administered OvuGel®.

There was no effect ($P > 0.05$) of Insemination Program treatment on the number of piglets born dead or born mummified in the current study. These results are to be expected, as Insemination Program would not be likely to have had a direct impact on either of these measures.

In conclusion, Insemination Program treatment impacted fertility in gilts that were administered Matrix®. Gilts administered OvuGel® on d 5 after the end of Matrix® administration had decreased pregnancy and farrowing rates compared to gilts administered OvuGel® on d 6 after Matrix® administration and Control gilts. When evaluating pregnancy and farrowing rate as a percentage of all gilts that were allotted to the study, OV6 gilts had similar pregnancy and farrowing rates compared to the Control, but when only evaluating Control gilts that expressed estrus and were inseminated, OV6 gilts had decreased pregnancy and farrowing rates compared to the Control gilts. Control gilts had a higher total number of piglets born than

OV5 gilts, and a higher number of piglets born alive than both timing of OvuGel® treatments. Although there was no statistical difference in the total number of piglets born between Control and OV6 gilts, it should be noted that there was a numeric decrease of 0.7 total piglets born between the Control and OV6 gilts, which could be economically important. Further research is necessary to determine the impact of administering OvuGel® at different time points and at different times of insemination following OvuGel® administration than those that were evaluated in the current study on both pregnancy and farrowing rates and litter size. There also may be merit in determining the impact of giving multiple inseminations to gilts administered OvuGel® on pregnancy and farrowing rates and litter size, however, this approach would obviously negate one of the potential benefits of this technology.

LITERATURE CITED

Almeida, F. R. C. L., S. Novak, G. R. Foxcroft. 2000. The time of ovulation in relation to estrus duration in gilts. Therio. 53: 1389-1396.

Bortolozzo, P. F., D. A. Uemoto, P. E. Bennemann, W. Barioni Jr., and I. Wentz. 2005. Influence of time of insemination relative to ovulation and frequency of insemination on gilt fertility. Therio. 64: 1956-1962.

Bracken, C. J., T. J. Safranski, T. C. Cantley, M. C. Lucy, and W. R. Lamberson. 2003. Effect of time of ovulation and sperm concentration on fertilization rate in gilts. Therio. 60: 669-676.

Davis, D. L., J. S. Stevenson, D. S. Pollman and G. L. Allee. 1987. Estrous and litter traits in gilts altered by altrenogest, flushing, and pubertal status. J. Anim. Sci. 64: 1117-1126.

Eliasson, L. 1989. A study on puberty and oestrus in gilts. J. Vet. Med. 36: 46-54.

Horsley, B. R., M. J. Estienne, A. F. Harper, S. H. Purcell, H. K. Baitis, W. E. Beal, and J. W. Knight. 2005. Effect of P.G. 600 on the timing of ovulation in gilts treated with altrenogest. J. Anim. Sci. 83: 1690-169.

Johnston, M. E., M. E. Swanson, and S. K. Webel. 2013a. (unpublished raw data).

Johnston, M. E., M. E. Swanson, and S. K. Webel. 2013b. (unpublished raw data).

Johnston, M. E., M. E. Swanson, and S. K. Webel. 2013c. (unpublished raw data).

Knox, R.V., K. L. Willenburg, S. L Rodriguez-Zas, D. L. Greger, H. D. Hafs, and M. E. Swanson. 2011. Synchronization of ovulation and fertility in weaned sows treated with intravaginal triptorelin is influenced by timing of administration and follicle size. Therio. 75: 308-319.

Littell, R. C., G. A. Milliken, W. W. Stroup, and R. D. Wolfinger. 1996. SAS systems for mixed models. SAS Inst. Inc., Cary, NC.

Nissen, A. K., N. M. Soede, P. Hyttel, M. Schmidt, and L. D'Hoore. 1997. The influence of time of insemination relative to time of ovulation on farrowing frequency and litter size in sows, as investigated by ultrasonography. Therio. 47:1571-1582.

NRC. 2012. Nutrient Requirements of Swine. 10th rev. ed. Natl. Acad. Press, Washington, DC.

Rhodes, M. T., D. L. Davis and J. S. Stevenson. 1991. Flushing and altrenogest affect litter traits in gilts. J. Anim. Sci. 69:34-40.

Soede, N. M., C. C. H. Wetzels, W. Zonda, M. A.I. de Koning and B. Kemp. 1995. Effects of time of insemination relative to ovulation, as determined by ultrasonography, on fertilization rate and accessory sperm count in sows. J. Reprod. Fertil. 104: 99-106.

Soede, N. M. and B. Kemp. 1997. Expression of oestrus and timing of ovulation in pigs. J. Reprod. Fertil. (Suppl. 52): 91-103.

Steverink, D. W. B., N. M. Soede, E. G. Bouwan and B. Kemp. 1997. Influence of insemination-ovulation interval and sperm cell dose on fertilization in sows. J. Reprod. Fert. 111: 165-171.

Steverink, D. W. B., N. M. Soede, G. J. R. Groenland, F. W. van Schie, J. P. T. M. Noordhuizen, and B. Kemp. 1999. Duration of estrus in relation to reproduction results in pigs on commercial farms. J. Anim. Sci. 77: 801-809.

Waberski, D., K. F. Weitze, T. Gleumes, M. Schwarz, T. Willmen and R. Petzoldt. 1994. Effect of time of insemination relative to ovulation on fertility with liquid and frozen boar semen. Therio. 42: 831-840.

TABLES

Table 2.1. Effect of timing of Insemination Program on gilt live weight prior to Matrix® administration and stage of estrous cycle prior to Matrix® administration (least-squares means), timing of estrus (least-squares means and percentage of the total), and number of inseminations per gilt (least-squares means).

	Insemination Program				
Item	Control	OV5	OV6	SEM	*P*-value
Number of gilts	149	146	143		
Gilt live weight (2 d prior to start of Matrix® administration), kg	146.7	147.4	147.3	1.11	0.71
Day of estrous cycle at start of Matrix® administration	9.5	9.7	9.7	0.46	0.72
Percentage exhibiting estrus from d 4 to 10 after Matrix® administration	81.9[a]	61.6[c]	72.2[b]	.	0.001
Percentage exhibiting behavioral estrus within day[1]					
Day 4	2.0	0.7	2.8	.	0.40
Day 5	17.5	20.6	20.8	.	0.72
Day 6	27.5	32.2	27.1	.	0.57
Day 7	20.8[a]	3.4[b]	20.1[a]	.	<0.001
Day 8	8.7[a]	2.1[b]	0.7[b]	.	0.001
Day 9	3.4	2.7	0.0	.	0.10
Day 10	2.0	0.0	0.7	.	0.18
Cumulative percentage exhibiting behavioral estrus[1]					
Day 4	2.0	0.7	2.8	.	0.40
Day 5	19.5	21.2	23.6	.	0.68
Day 6	47.0	53.4	50.7	.	0.54
Day 7	67.8[a]	56.9[b]	70.8[a]	.	0.03
Day 8	76.5[a]	58.9[b]	71.5[a]	.	0.004
Day 9	79.9[a]	61.6[bc]	71.5[ab]	.	0.002
Day 10	81.9[a]	61.6[b]	72.2[b]	.	0.001
Time interval from end of Matrix® administration to:					
Start of estrus, h[3]	151.3[a]	140.0[b]	140.9[b]	2.51	0.002
End of estrus, h[4]	196.7[a]	179.3[c]	188.2[b]	2.07	<0.001
Estrus duration, h[5]	49.1[a]	41.1[bc]	47.3[ab]	1.38	0.001
Percentage exhibiting estrus at time of insemination[6]	100.0[a]	40.4[c]	64.6[b]	.	<0.001
Number of inseminations per gilt	1.98[a]	1.00[b]	1.00[b]	0.013	<0.001

[a,b,c]Means within a row with different superscripts differ ($P < 0.05$).
[1]Days after Matrix® administration was concluded.
[2]Hour 0 = Hour last dose of Matrix® was administered. Matrix® was administered daily at 0900 and estrus detection was completed daily at 0630 and 1830 (beginning on d 4 after the last dose of Matrix® was administered.
[3]First time behavioral estrus was exhibited minus 6 h.
[4]First time behavioral estrus was not exhibited minus 6 h.
[5]From (first time behavioral estrus was exhibited minus 6 h) to (first time behavioral estrus was not exhibited minus 6 h).
[6]Measured as a percentage of gilts inseminated.

Table 2.2. Effect of Insemination Program on the percentage of gilts exhibiting estrus, the least-squares means for the time interval from the end of Matrix® administration to the start and end of estrus, estrus duration, time interval from start of estrus to ovulation, timing of ovulation as a percentage of estrus duration, and least-squares means for single ovary follicle number and size prior to ovulation.[1]

	Insemination Program				
Item	Control	OV5	OV6	SEM	*P*-value
Number of gilts	51	38	50		
Percentage exhibiting estrus between d 4-10 after Matrix® administration	79.5[a]	57.1[b]	67.5[ab]	.	0.008
Time interval from end of Matrix® administration to:[2]					
Start of estrus, h[3]	152.5[a]	143.6[b]	139.3[b]	3.00	0.003
End of estrus, h[4]	202.1[a]	179.2[c]	187.2[b]	2.75	<0.001
Estrus duration, h[5]	49.1[a]	37.0[b]	46.5[a]	1.87	<0.001
Time interval from start of estrus to ovulation, h[3, 6]	34.2[a]	26.0[b]	34.0[a]	1.51	0.001
Time interval to ovulation as a percentage of estrus duration[6,7]	68.6	74.3	76.4	2.52	0.06
Follicles:[8]					
Number 12 h prior to ovulation	10.5	10.6	10.5	0.31	0.96
Size 12 h prior to ovulation, mm	6.6[a]	6.1[b]	6.4[a]	0.09	0.001

[a,b,c]Means within a row with different superscripts differ ($P \leq 0.05$).

[1]A subsample of gilts were ultrasonically scanned to determine the timing of ovulation.

[2]Hour 0 = Hour last dose of Matrix® was administered. Matrix® was administered daily at 0900 and estrus detection was completed daily at 0630 and 1830 (beginning on d 4 after the last dose of Matrix® was administered).

[3]First time behavioral estrus was exhibited minus 6 h.

[4]First time behavioral estrus was not exhibited minus 6 h.

[5]From (first time behavioral estrus was exhibited minus 6 h) to (first time behavioral estrus was not exhibited minus 6 h).

[6]Defined for each gilt as: time interval from start of estrus to ovulation divided by estrus duration.

[7]The time of ovulation (ovulation h) was measured as the time ovulation was completed (i.e. fewer than 4 follicles remaining on the ovary) minus 6 h.

[8]One ovary from each gilt was scanned in the determination of ovulation and follicle number and size.

Table 2.3. Effect of Insemination Program on the timing of ovulation within day and cumulatively after the end of Matrix® administration.[1]

	Insemination Program				
Item	Control	OV5	OV6	SEM	*P*-value
Number of gilts	67	79	69		
Percentage ovulating within 10 d after Matrix® administration	88.1	84.8	95.7	.	0.10
Percentage ovulating within day, %[2]					
Day 4	0.0	0.0	0.0	.	.
Day 5	0.0	0.0	2.9	.	0.12
Day 6	6.0	2.5	2.9	.	0.50
Day 7	22.4[c]	74.7[a]	37.7[b]	.	<0.001
Day 8	37.3[a]	3.8[b]	52.2[a]	.	<0.001
Day 9	16.4[a]	1.3[b]	0.0[b]	.	<0.001
Day 10	6.0	2.5	0.0	.	0.11
Cumulative percentage ovulating, %[2]					
Day 4	0.0	0.0	0.0	.	.
Day 5	0.0	0.0	2.9	.	0.12
Day 6	6.0	2.5	5.8	.	0.53
Day 7	28.4[b]	77.2[a]	43.5[b]	.	<0.001
Day 8	65.7[c]	81.0[b]	95.7[a]	.	<0.001
Day 9	82.1[b]	82.3[b]	95.7[a]	.	0.03
Day 10	88.1	84.8	95.7	.	0.10

[a,b,c]Means within a row with different superscripts differ ($P < 0.05$).

[1]A subsample of gilts were ultrasonically scanned to determine the timing of ovulation.

[2]Days after Matrix® administration was concluded.

Table 2.4. Effect of timing of OvuGel® administration on the least-squares means for the time interval from the end of Matrix® administration to ovulation, OvuGel® administration to ovulation, insemination to ovulation; and the percentage ovulating after OvuGel® administration and insemination.[1]

	OvuGel® Timing			
Item	OV5	OV6	SEM	*P*-value
Number of gilts	67	66		
Time interval from, h:[2]				
End of Matrix® administration to ovulation[3]	164.8[b]	174.2[a]	2.17	<0.001
OvuGel® administration to ovulation, h	46.8[a]	32.2[b]	1.84	<0.001
Insemination to ovulation, h	22.8[a]	8.2[b]	1.84	<0.001
Ovulation prior to insemination[4]	.	13.2	.	.
Ovulation after insemination	22.8[a]	14.5[b]	1.60	0.001
Percentage ovulating:[2]				
Within 48 h after OvuGel® administration	70.9[b]	92.5[a]	.	0.001
Within 24 h after insemination	70.9	75.4	.	0.54

[a,b,c]Means within a row with different superscripts differ ($P < 0.05$).

[1]A subsample of gilts were ultrasonically scanned to determine the timing of ovulation.

[2]The time of ovulation (ovulation h) was measured as the time ovulation was completed (i.e. fewer than 4 follicles remaining on the ovaries) minus 6 h.

[3]Time interval from end of Matrix® administration to ovulation for Control gilts = 189.7 h; Control gilts were included in the analysis and had a greater ($P \leq 0.05$) time interval from the end of Matrix® administration to ovulation compared to OV5 and OV6.

[4]OV6 was the only treatment in which any gilts ovulated prior to insemination (n = 15), therefore the mean of the interval of these animals is presented.

Table 2.5. Effect of Insemination Program treatment on pregnancy and farrowing rates, gestation length, and litter size.[1]

	Insemination Program				
Item	Control	OV5	OV6	SEM	*P*-value
Number of gilts:					
Allotted to study[2]	149	146	143		
Inseminated	122	146	143		
Pregnant	111	83	112		
Pregnancy rate, %					
Of gilts allotted to the study[2]	74.5[a]	56.9[b]	79.0[a]	.	<0.001
Of gilts inseminated	91.0[a]	56.9[c]	79.0[b]	.	<0.001
Farrowing rate, %					
Of gilts allotted to the study[2]	73.8[a]	55.5[b]	78.3[a]	.	<0.001
Of gilts inseminated	90.2[a]	55.5[c]	78.3[b]	.	<0.001
Number of gilts:					
Exhibiting estrus at insemination	122	59	93		
Not exhibiting estrus at insemination	.	87	50		
Pregnant, estrus at insemination	111	44	85		
Pregnant, no estrus at insemination	.	39	28		
Farrowing, estrus at insemination	110	44	85		
Farrowing, no estrus at insemination	.	37	27		
Pregnancy rate, %					
Gilts exhibiting estrus at insemination	91.0[a]	74.6[b]	91.4[a]	.	0.003
Gilts not exhibiting estrus at insemination	.	44.8	56.0	.	0.21
Farrowing rate, %					
Gilts exhibiting estrus at insemination	90.2[a]	74.6[b]	91.4[a]	.	0.004
Gilts not exhibiting estrus at insemination	.	42.5	54.0	.	0.20
Gestation length, d[3]	115.3	115.3	115.1	0.11	0.21
Average litter size:					
Total born	13.2[a]	12.1[b]	12.6[ab]	0.31	0.05
Born alive	12.5[a]	11.2[b]	11.7[b]	0.22	0.01
Born dead[4]	0.2	0.4	0.3	0.06	0.09
Mummified[4]	0.2	0.3	0.3	0.06	0.86

[a,b,c]Means within a row with different superscripts differ ($P < 0.05$).

[1]Gestation length and litter size were analyzed using least-squares means.

[2]Allotment to study occurred at the start of Matrix® administration.

[3]For the Control treatment, the first day of insemination was used in the calculation of gestation length.

[4]Data were transformed using the PROC RANK procedure of SAS.

FIGURES

Figure 2.1. Frequency distribution for the time interval from the end of Matrix® administration to ovulation.

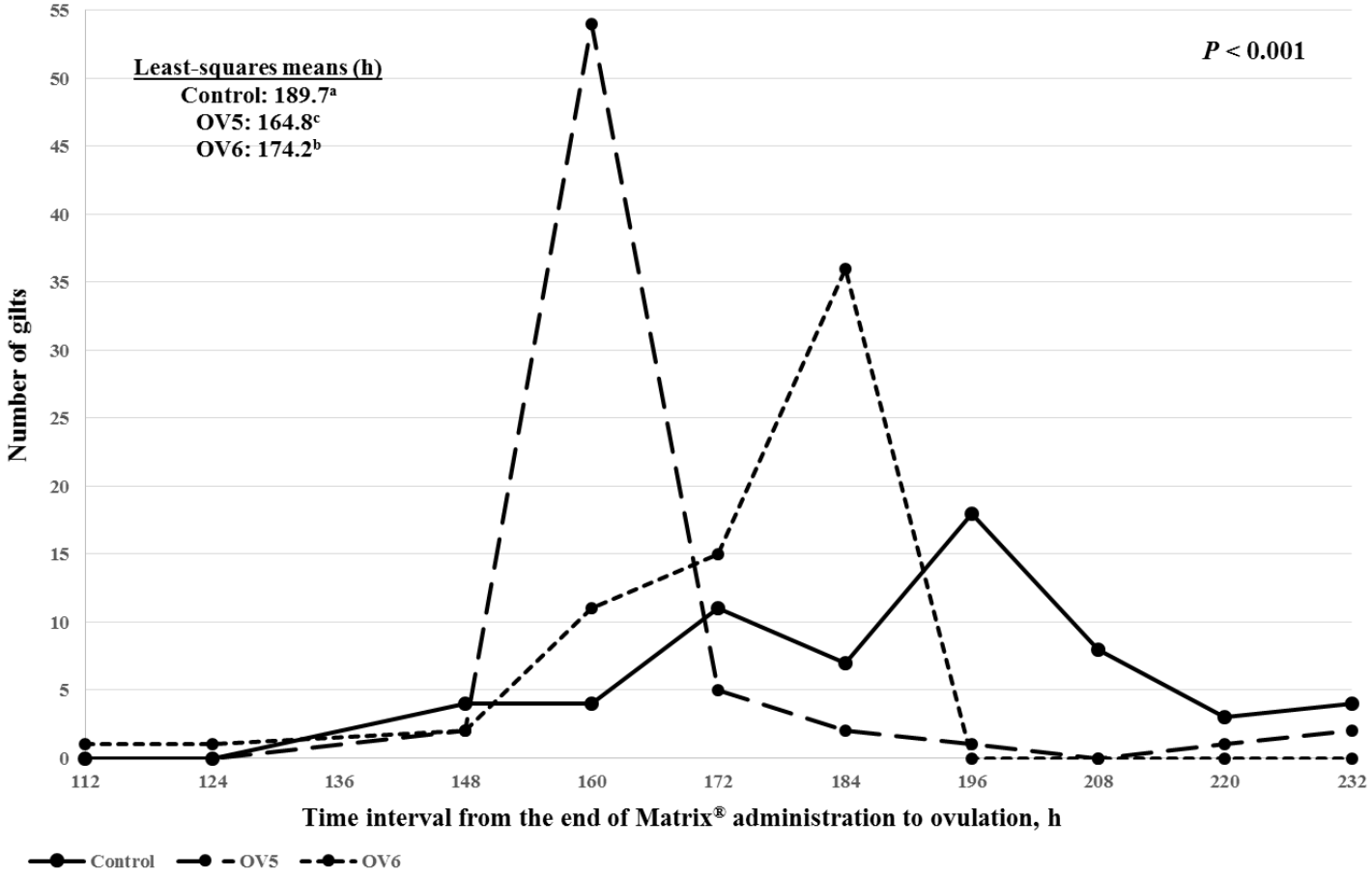


Figure 2.2. Frequency distribution for the time interval from OvuGel® administration to ovulation.

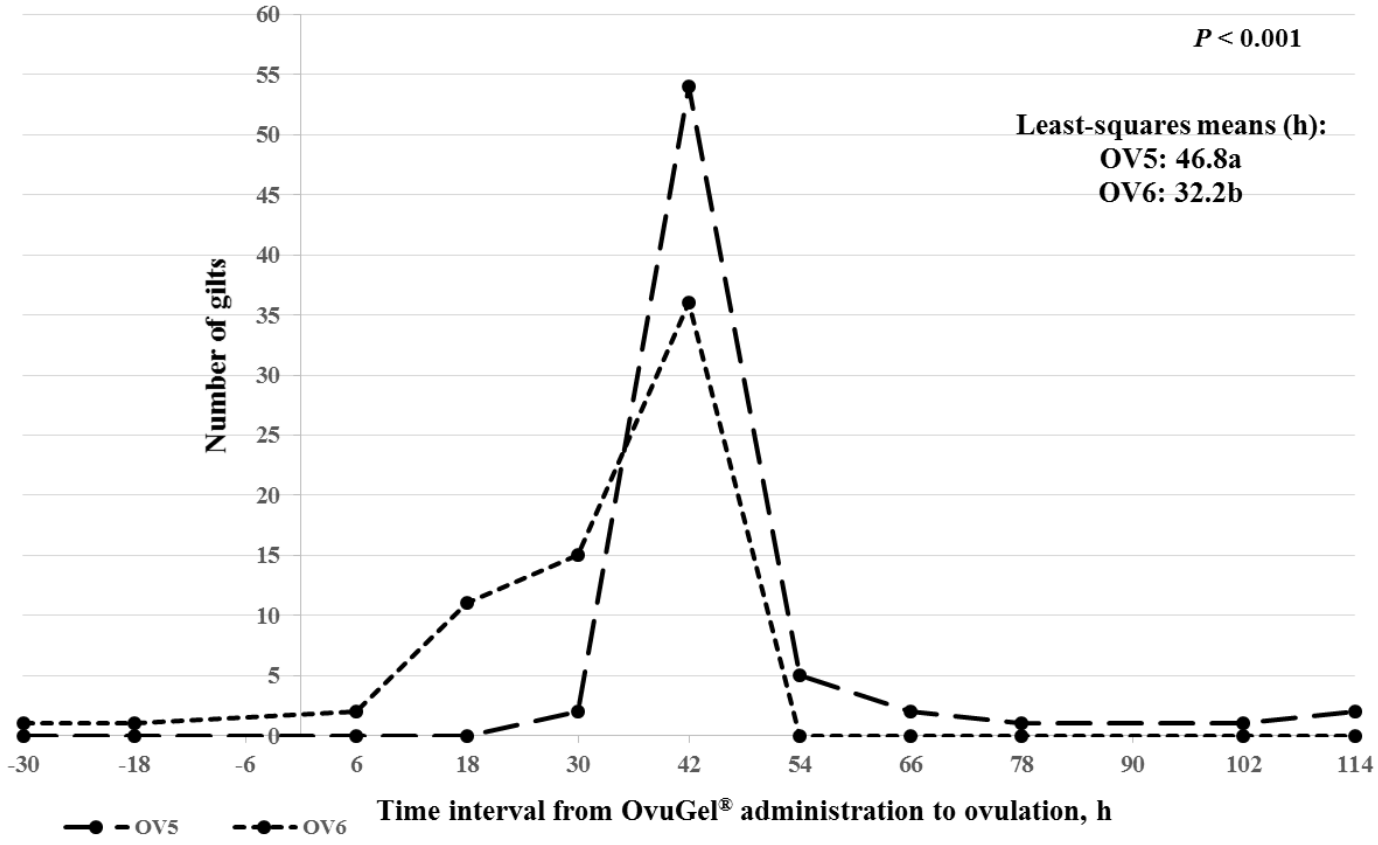
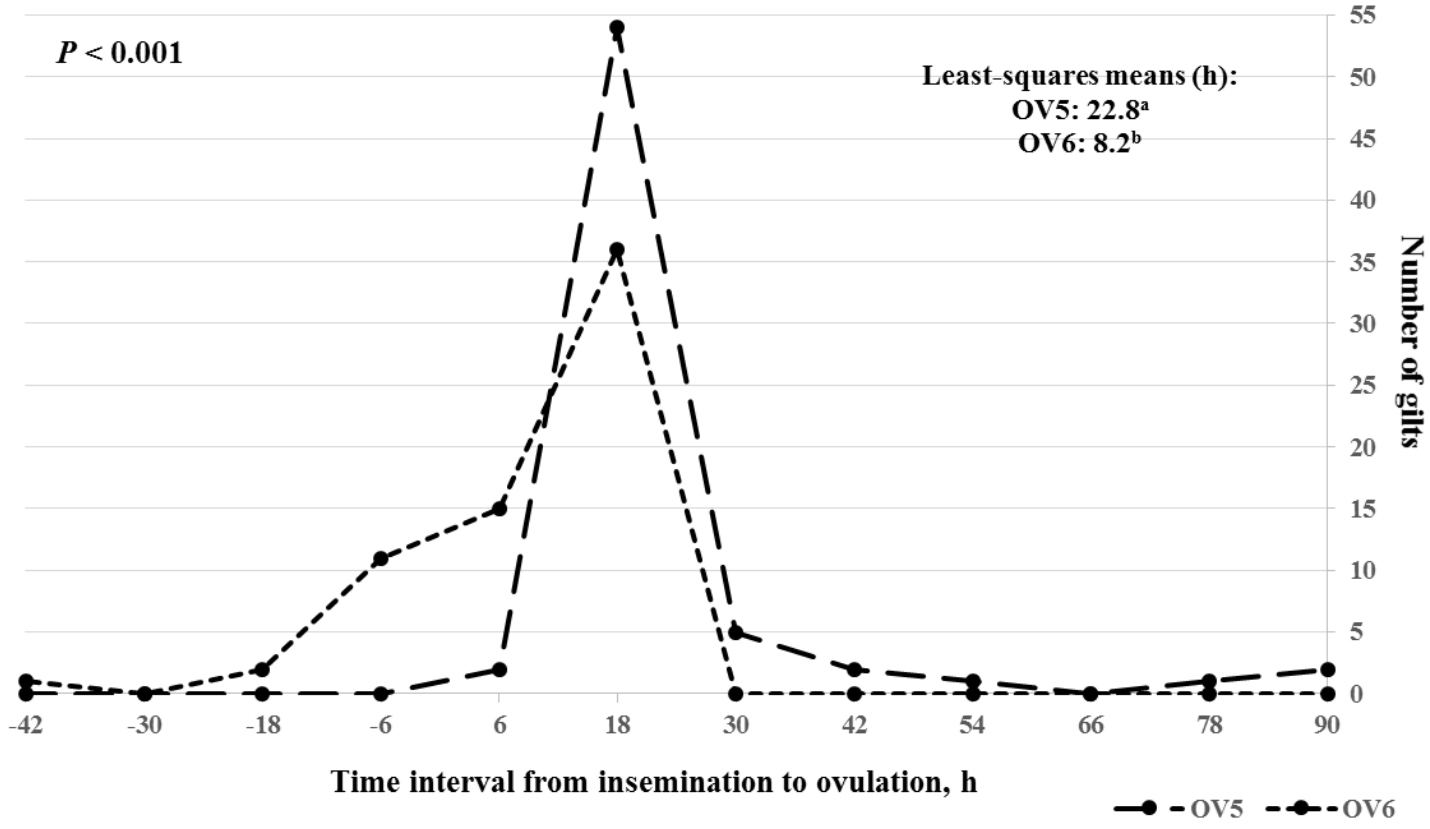


Figure 2.3. Frequency distribution for the time interval from insemination to ovulation (for OvuGel® treated gilts).



APPENDIX A: TABLES

Table A.1. Descriptive statistics for estrus and insemination measures.

		Descriptive statistics			
Item	N	Mean	Standard Deviation	Minimum	Maximum
Gilt live weight (2 d prior to start of Matrix® administration)	438	324.0	29.98	260.0	406.0
Day of estrous cycle at start of Matrix® administration	439	9.7	5.67	-1.0	21.0
Time interval to estrus after Matrix® administration, h[1]					
Start[2]	316	144.3	26.14	88.0	244.0
End[3]	309	188.7	21.75	136.0	256.0
Duration[4]	310	46.2	14.34	12.0	72.0
Number of inseminations per female	411	1.29	0.469	1.00	3.00

[1]Hour 0 = Hour last dose of Matrix® was administered. Matrix® was administered daily at 0900 and estrus detection was completed daily at 0630 and 1830 (from d 4 to 10 after the last dose of Matrix® was administered).

[2]First time behavioral estrus was exhibited minus 6 h.

[3]First time behavioral estrus was not exhibited minus 6 h.

[4]From (first time behavioral estrus was exhibited minus 6 h) to (first time behavioral estrus was not exhibited minus 6 h).

Table A.2. Descriptive statistics for estrus and ultrasound measures.[1]

		Descriptive statistics			
Item	N	Mean	Standard Deviation	Minimum	Maximum
Time interval from end of Matrix® administration to:[2]					
Start of estrus[3]	170	146.6	25.04	88.0	244.0
End of estrus[4]	168	190.7	22.47	136.0	256.0
Estrus duration[5]	168	45.1	14.7	12.0	72.0
Time interval to ovulation after Matrix® administration, h[2,6]	192	175.7	20.01	112.0	232.0
Time interval to ovulation after start of estrus, h[3,6,7]	139	32	10.85	12.0	60.0
Interval from OvuGel® administration to ovulation[6]	133	39.6	16.60	-30.0	114.0
Interval from ovulation to insemination, h[6]	133	15.6	16.63	-54.0	90.0
Ovulation time as a percentage of estrus duration[5,6]	139	72.9	17.30	20.0	100.0
Single ovary follicle number 12 h prior to ovulation[8]	168	10.5.	2.40	4.0	18.0
Single ovary follicle size 12 h prior to ovulation, mm[8]	167	6.4	0.74	4.7	9.1
Single ovary follicle number[8]					
Day[9]					
4	165	13.3	2.91	7.0	21.0
5	172	12.8	2.84	6.0	21.0
6	219	11.6	2.45	5.0	19.0
7	152	11.1	2.34	6.0	18.0
8	56	11.2	2.91	6.0	18.0
9	32	11.2	2.91	7.0	21.0
10	18	11.8	3.26	7.0	21.0
Single ovary follicle size[8]					
Day[9]					
4	165	4.9	0.64	3.2	6.2
5	172	5.7	0.7	3.8	7.2
6	218	6.2	0.71	4.0	7.7
7	150	6.3	0.7	4.4	7.6
8	52	6.3	0.98	4.3	8.3
9	30	6.2	1.22	3.7	9.0
10	18	6.3	1.29	4.3	9.1

[1]Measured on a subsample of approximately 65 gilts per treatment.
[2]Hour 0 = Hour last dose of Matrix® was administered. Matrix® was administered daily at 0900 and estrus detection was completed daily at 0630 and 1830 (beginning on d 4 after the last dose of Matrix® was administered).
[3]First time behavioral estrus was exhibited minus 6 h.
[4]First time behavioral estrus was not exhibited minus 6 h.
[5]From (first time behavioral estrus was exhibited minus 6 h) to (first time behavioral estrus was not exhibited minus 6 h).
[6]The time of ovulation (ovulation h) was measured as the time ovulation was completed (i.e. fewer than 4 follicles remaining on the ovaries) minus 6 h.
[7]Hour 0 = first time behavioral estrus was exhibited minus 6 h.
[8]Follicle number and size were calculated as the mean of both measurements taken each day (at 0430 and 1630).
[9]Days after the end of Matrix® administration.

Table A.3. Descriptive statistics for gestation length and litter size measures.

		Descriptive statistics			
Item	N	Mean	Standard Deviation	Minimum	Maximum
Gestation length, d[1]	303	115.3	1.07	110.0	118.0
Litter size					
Total born	299	12.6	3.16	4.0	20.0
Born alive	299	11.9	3.00	3.0	18.0
Born dead	295	0.3	0.57	0.0	2.0
Mummified	288	0.3	0.60	0.0	2.0

[1]For the Control treatment, the first day of insemination was used in the calculation of gestation length.

Table A.4. Effect of Insemination Program on the percentage of gilts exhibiting behavioral estrus each 12 h interval and cumulatively after Matrix® administration.[1]

	Insemination Program			
Item	Control	OV5	OV6	*P*-value
Percentage exhibiting behavioral estrus within day, %[2]				
Day 4				
AM	0.7	0.7	1.4	0.76
PM	1.3	0.0	1.4	0.37
Day 5				
AM	8.1	6.9	6.3	0.83
PM	9.4	13.7	14.6	0.36
Day 6				
AM	16.1	20.6	13.2	0.24
PM	11.4	11.6	13.9	0.78
Day 7				
AM	13.4[a]	2.1[b]	18.8[a]	<0.001
PM	7.4[a]	1.4[b]	1.4[b]	0.01
Day 8				
AM	6.7[a]	0.7[b]	0.0[b]	0.01
PM	2.0	1.4	0.7	0.62
Day 9				
AM	2.7	1.4	0.0	0.14
PM	0.7	1.4	0.0	0.37
Day 10				
AM	0.7	0.0	0.7	0.61
PM	1.3	0.0	0.0	0.14
Cumulative percentage exhibiting behavioral estrus, %[2]				
Day 4				
AM	0.7	0.7	1.4	0.76
PM	2.0	0.7	2.8	0.40
Day 5				
AM	10.1	7.5	9.0	0.74
PM	19.5	21.2	23.6	0.68
Day 6				
AM	35.6	41.8	36.8	0.51
PM	47.0	53.4	50.7	0.54
Day 7				
AM	60.4[ab]	55.5[b]	69.4[a]	0.05
PM	67.8[a]	56.9[b]	70.8[a]	0.03
Day 8				
AM	74.5[a]	57.5[b]	70.8[a]	0.01
PM	76.5[a]	58.9[b]	71.5[a]	0.004
Day 9				
AM	79.2[a]	60.3[b]	71.5[a]	0.002
PM	79.9[a]	61.6[b]	71.5[a]	0.003
Day 10				
AM	80.5[a]	61.6[b]	72.2[a]	0.002
PM	81.9[a]	61.6[c]	72.2[b]	0.001

[a,b,c]Means within a row with different superscripts differ ($P \leq 0.05$).

[1]Gilts were checked for behavioral estrus at 12 h intervals (0630 and 1830) from d 4 to 10 after the end of Matrix® administration.

[2]Days after the end of Matrix® administration.

Table A.5. Effect of Insemination Program on the percentage of gilts ovulating each 12 h interval and cumulatively after Matrix® administration.[1,2]

	Insemination Program			
Item	Control	OV5	OV6	*P*-value
Percentage ovulating, within day %[3]				
Day 4				
AM	0.0	0.0	0.0	.
PM	0.0	0.0	0.0	.
Day 5				
AM	0.0	0.0	1.5	0.35
PM	0.0	0.0	1.5	0.35
Day 6				
AM	0.0	0.0	0.0	.
PM	6.0	2.5	2.9	0.5
Day 7				
AM	6.0[b]	68.4[a]	15.9[b]	<0.001
PM	16.4[a]	6.3[b]	21.7[a]	0.02
Day 8				
AM	10.5[b]	2.5[c]	52.2[a]	<0.001
PM	26.9[a]	1.3[b]	0.0[b]	<0.001
Day 9				
AM	11.9[a]	0.0[b]	0.0[b]	0.01
PM	4.5	1.3	0.0	0.14
Day 10				
AM	6.0	2.5	0.0	0.11
PM	.	.	.	.
Cumulative percentage ovulating, %[3]				
Day 4				
AM	0.0	0.0	0.0	.
PM	0.0	0.0	0.0	.
Day 5				
AM	0.0	0.0	1.5	0.35
PM	0.0	0.0	2.9	0.12
Day 6				
AM	0.0	0.0	2.9	0.12
PM	6.0	2.5	5.8	0.53
Day 7				
AM	11.9[b]	70.9[a]	21.7[b]	<0.001
PM	28.4[b]	77.2[a]	43.5[b]	<0.001
Day 8				
AM	38.8[c]	79.8[a]	95.7[a]	<0.001
PM	65.7[c]	81.0[b]	95.7[a]	<0.001
Day 9				
AM	77.6[b]	81.0[b]	95.7[a]	0.01
PM	82.1[b]	82.3[b]	95.7[a]	0.03
Day 10				
AM	88.1	84.8	95.7	0.10
PM	.	.	.	.

[a,b,c]Means within a row with different superscripts differ ($P \leq 0.05$).

[1]A subsample of gilts were ultrasonically (trans-rectally) scanned (n = approximately 65 per treatment).

[2]Gilts were ultrasonically scanned at 12 h intervals (0430 and 1630) from d 4 to 10 after the end of Matrix® administration.

[3]Days after the end of Matrix® administration.

Table A.6. Effect of treatment and time on follicle number and size.[1]

	Insemination Program				Day[2]								*P*-value		
Item	Control	OV5	OV6	SEM	4	5	6	7	8	9	10	SEM	Treatment	Day	Treatment × Day
Follicle number[3]	11.6	12.1	11.8	0.30	13.4^{a}	12.7^{b}	11.6^{c}	11.1^{d}	11.2^{cd}	11.9^{bcd}	11.0^{cd}	0.34	0.38	<0.001	0.23
Follicle size[3]	6.1	5.9	6.2	0.09	5.1^{e}	5.8^{d}	6.1^{c}	6.1^{c}	6.1^{bc}	6.4^{d}	6.7^{a}	0.08	0.07	<0.001	<0.001
Day[2]															
4	5.2^{hi}	5.0^{i}	5.2^{h}	0.09											
5	5.9^{f}	5.5^{g}	5.9^{f}	0.09											
6	6.2^{bcde}	5.8^{f}	6.2^{cde}	0.08											
7	6.3^{bcd}	5.8^{f}	6.3^{bcde}	0.10											
8	6.3^{bcd}	6.0^{ef}	6.2^{bcdef}	0.17											
9	6.4^{bcd}	6.6^{b}	6.2^{bcdef}	0.19											
10	6.0^{def}	6.6^{bc}	7.4^{a}	0.23											

^{a,b,c,d,e,f,g,h,i}Means within a row with different superscripts differ ($P < 0.05$).

[1]Measured on a subsample of approximately 65 gilts per treatment.

[2]Days after the end of Matrix® administration.

[3]Follicle number and size were calculated as the mean of both measurements taken within a day (at 0430 and 1630).

Table A.7. Effect of timing of OvuGel® administration on the pregnancy outcome within each insemination to ovulation interval.

	OV5[2]		OV6[2]	
Insemination to ovulation interval (h)[1]	Number Pregnant	Number Not Pregnant	Number Pregnant	Number Not Pregnant
-42	0	0	0	1
-30	0	0	0	0
-18	0	0	1	1
-6	0	0	8	3
6	2	0	12	3
18	39	15	31	5
30	1	4	0	0
42	1	1	0	0
54	1	0	0	0
66	0	0	0	0
78	0	1	0	0
90	0	2	0	0

[1]Negative insemination to ovulation interval equates to ovulation occurring prior to insemination; hour 0 = time of insemination.
[2]Reported as number of gilts.

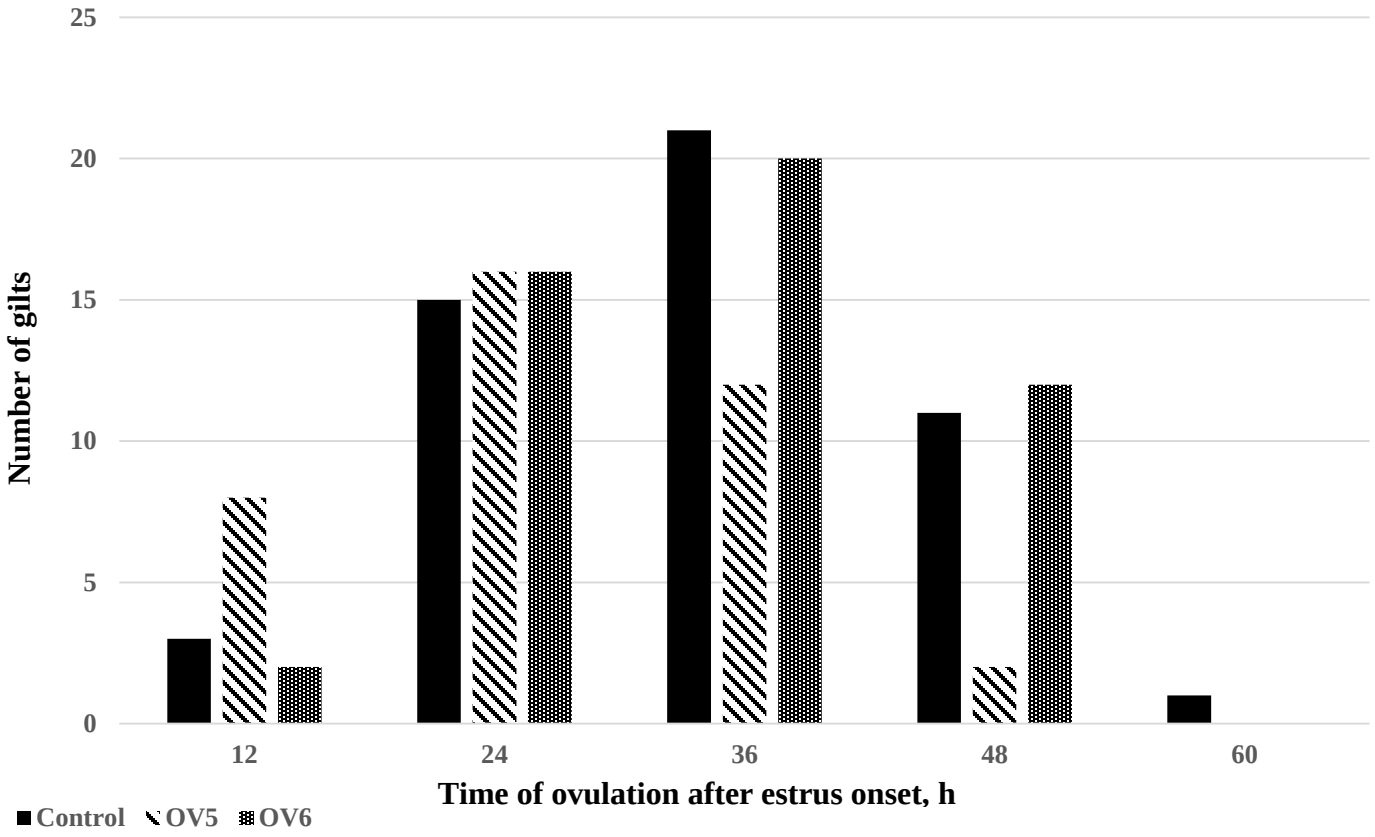
Table A.8. Effect of Insemination Program on the pregnancy rate within each insemination to ovulation interval.

	Pregnancy Rate, %[2]	
Insemination to ovulation interval (h)[1]	OV5	OV6
-42	-	0.0
-30	-	-
-18	-	50.0
-6	-	72.7
6	100.0	80.0
18	72.2	86.1
30	20.0	-
42	50.0	-
54	100.0	-
66	-	-
78	0.0	-
90	0.0	-

[1]Negative insemination to ovulation interval equates to ovulation occurring prior to insemination; hour 0 = time of insemination.
[2]Calculated as the number of gilts pregnant from one insemination to ovulation interval divided by the total number of gilts representing that insemination to ovulation interval (numbers presented in Appendix Table 2.7).

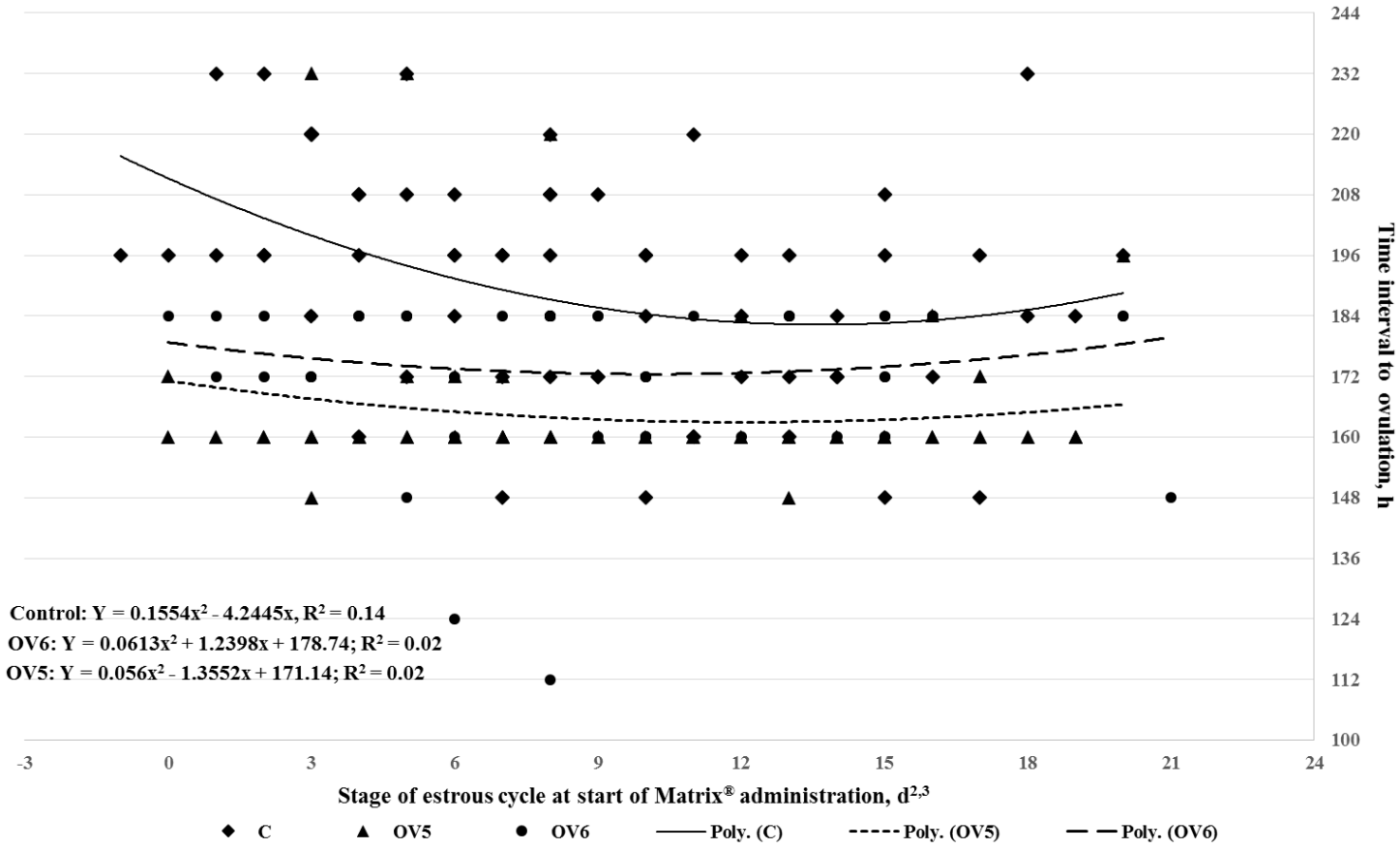
APPENDIX B: FIGURES

Figure B.1. Frequency distribution of the timing of ovulation after onset of estrus.[1,2]



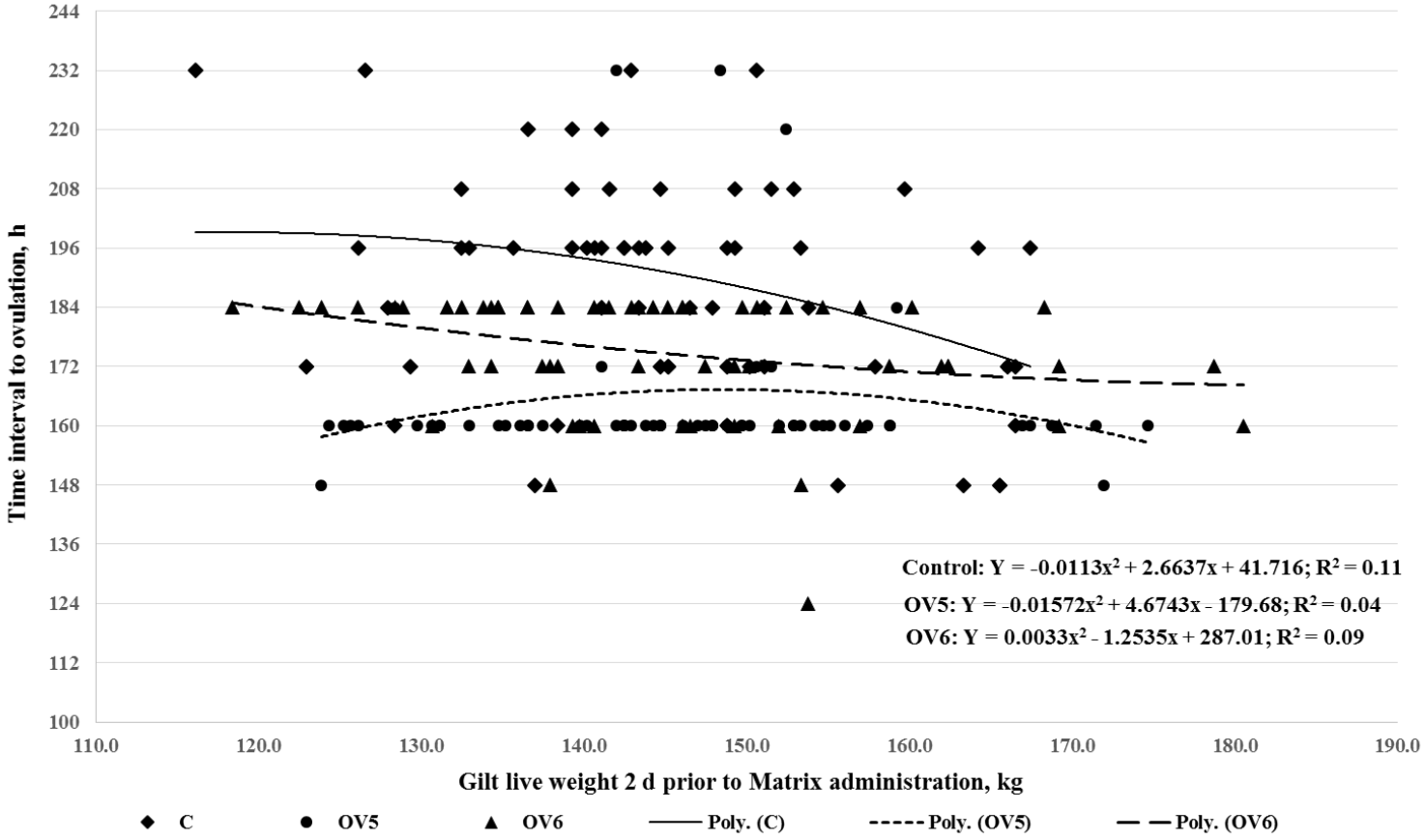
[1]Gilts were ultrasonically scanned at 12 h intervals (0430 and 1630) from d 4 to 10 after the end of Matrix® administration.
[2]Gilts administered OvuGel were inseminated regardless of the expression of behavioral estrus.

Figure B.2. Relationship between the stage of estrous cycle prior to the start of Matrix® administration and the time interval from the end of Matrix® administration to ovulation.[1]



[1]Gilts were ultrasonically scanned at 12 h intervals (0430 and 1630) from d 4 to 10 after the end of Matrix® administration.
[2]Days since last estrus occurrence.
[3]Day 0 = first estrus occurrence on the first day of Matrix® administration

Figure B.3. Relationship between gilt live weight prior at the start of Matrix® administration and the time interval from the end of Matrix® administration to ovulation.[1]



[1]Gilts were ultrasonically scanned at 12 h intervals (0430 and 1630) from d 4 to 10 after the end of Matrix® administration.