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Effect of time of ovulation and sperm concentration on fertilization rate in gilts

C.J. Bracken, T.J. Safranski, T.C. Cantley, M.C. Lucy, W.R. Lamberson*

Department of Animal Science, Animal Sciences Unit, University of Missouri, 159 Animal Sciences Center, Columbia, MO 65211, USA

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Abstract

In normal production practices, sows and gilts are inseminated at least twice during estrus because the timing of ovulation is variable relative to the onset of estrus. The objective of this study was to determine if a normal fertilization rate could be achieved with a single insemination of low sperm number given at a precise interval relative to ovulation. Gilts ($n = 59$) were randomly assigned to one of three treatment groups: low dose (LD; one insemination, 0.5×10^9 spermatozoa), high dose (HD; one insemination, 3×10^9 spermatozoa) or multiple dose (MD; two inseminations, 3×10^9 spermatozoa per insemination). Twice daily estrus detection (06:00 and 18:00 h) was performed using fenceline boar contact and backpressure testing. Transrectal ultrasonography was performed every 6 h beginning at the detection of the onset of standing estrus and continuing until ovulation. Gilts in the LD and HD groups were inseminated 22 h after detection of estrus; MD gilts received inseminations at 10 and 22 h after detection of estrus. Inseminations were administered by using an insemination catheter and semen was deposited into the cervix. The uterus was flushed on Day 5 after the onset of estrus and the number of corpora lutea, oocytes, and embryos were counted. Time of insemination relative to ovulation was designated as 40 to >24 h, 24 to >12 h, and 12 to 0 h before ovulation and >0 h after ovulation. The LD gilts had fewer embryos ($P < 0.04$), more unfertilized oocytes ($P < 0.05$) and a lower fertilization rate ($P < 0.07$) compared to MD gilts. The effects of time of insemination relative to ovulation and the treatment by time interaction were not significant. We conclude that a cervical insemination with low spermatozoa concentration may not result in acceptable fertility even when precisely timed relative to ovulation.
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Keywords: Gilt; Estrus; Ovulation; Insemination dose; Time

* Corresponding author. Tel.: +1-573-882-8234; fax: +1-573-882-6827.
E-mail address: LambersonW@missouri.edu (W.R. Lamberson).

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1. Introduction

Conception and farrowing rates after artificial insemination in swine depend on the time of insemination relative to ovulation [1–3]. Multiple inseminations with greater than 2.5 billion spermatozoa have been employed as a standard swine practice because the time of ovulation relative to the onset of estrus is too imprecise to accurately time insemination relative to ovulation. Soede et al. [4] found that good fertility could be obtained when inseminations containing 3×10^9 spermatozoa were administered 24 h prior to ovulation. Maximum fertility occurred when inseminations were 8–12 h prior to ovulation. Nissen et al. [5] used inseminations containing 2×10^9 spermatozoa and reported good fertility when sows were inseminated within 28 h before ovulation. Similar results for gilts were found with maximum fertility occurring when 2×10^9 spermatozoa were inseminated within 24 h of ovulation [6,7].

The aforementioned studies [4–7] used transrectal ultrasonography to determine time of ovulation in sows and identified the optimum breeding window for high fertilization rates. Steverink et al. [8] tested 1×10^9–6×10^9 spermatozoa per insemination in sows and concluded that the number of inseminated spermatozoa did not affect the optimal breeding window (24 h before ovulation). In the present study, we examined a similar hypothesis for gilts and employed a lower sperm dose (0.5×10^9 spermatozoa per insemination). The objective was to determine if a normal fertilization rate could be achieved with a single insemination of low sperm number given at a precise interval relative to ovulation.

2. Materials and methods

2.1. Animals and estrus detection

Fifty-nine crossbred gilts were used. Estrus detection using fenceline boar contact and the backpressure test was started when gilts were approximately 180 days of age. Gilts were initially housed in one-quarter hectare pasture lots and then were placed in individual 1.2 m × 1.8 m indoor pens prior to the expected onset of their second estrus. Twice daily estrus detection at 06:00 and 18:00 h was initiated beginning 1–2 days before the expected date of second estrus. Estrus was defined as a standing reflex in the presence of the boar. Estrus detection continued until the standing reflex subsided.

2.2. Transrectal ultrasonography

Transrectal ultrasonography was performed with an Aloka 500 V ultrasound scanner (Corometrics Medical Systems, Inc., Wallingford, CT) and a 7.5 MHz linear transducer. The transducer was attached to a handle. Ultrasonography was initiated at the onset of standing estrus. The gilts were loaded into a gated crate and offered a small amount of feed. The transducer and handle were liberally coated with obstetrical lubricating jelly and then slowly and gently inserted into the rectum. The ovaries were examined and the presence or absence of preovulatory follicles (6–8 mm) was determined. Ultrasonography was performed once every 6 h until ovulation (the first observation of the absence of preovulatory follicles).

2.3. Artificial insemination

Gilts were randomly assigned to one of three insemination treatments: low dose (LD; one insemination of 0.5×10^9 spermatozoa), high dose (HD; one insemination of 3×10^9 spermatozoa) or multiple dose (MD; two inseminations of 3×10^9 spermatozoa per insemination). A higher variance for the average number of collected embryos was expected in LD gilts because of lower conception rate. Thus, twice as many gilts were initially assigned to the LD treatment so that standard errors would be approximately equal across treatments. In addition, MD gilts were added later in the experiment to confirm that acceptable embryo recovery occurred when industry insemination practices (two inseminations of 3×10^9 spermatozoa per insemination) were employed. Semen was collected from 1- to 2-year-old boars ($n = 9$) housed at the University of Missouri. An IMV Micro-Reader I spectrophotometer (IMV International, Inc., Minneapolis, MN) was used to determine sperm concentration of the semen. The semen was then extended to 80 ml for each treatment dose using a Modified Modena extender [9] and was used within 24 h of collection. A single semen collection was diluted so that it could be used to inseminate estrual gilts randomly assigned to different treatments. One technician performed the semen dilutions and inseminations for the entire experiment. Inseminations were administered by using a Goldenpig® insemination catheter (IMV International, Inc.) and semen was deposited into the cervix. The LD and HD treatment groups were inseminated once at 22 h after detection of estrus. Gilts assigned to the MD treatment were inseminated at 10 and 22 h after detection of estrus.

2.4. Embryo collection

Oocytes and embryos were collected on Day 5 after the onset of estrus by surgically flushing the uterus. Gilts were anesthetized with 5% sodium thiopental (0.15 ml/kg) and maintained with 2.5% halothane on a closed circuit system. The reproductive tract was exteriorized via mid-ventral laporatomy. Corpora lutea (CL) were counted to determine ovulation rate. A blunt incision was made in each uterine horn above the bifurcation and the flared end of a blown glass cannula was introduced. Approximately 30 ml of Hepes BSA media were injected at the uterotubal junction, massaged down the uterine horn and emptied from the uterus through the cannula into a sterile collection tube. The uterine flush was examined under a stereomicroscope and embryos as well as unfertilized oocytes (UFO) were counted.

2.5. Statistical analyses

Interval from insemination to ovulation for MD gilts was based on the second insemination time (22 h after detection of estrus). Time of insemination relative to ovulation was classified into one of four intervals (40 to >24 h before ovulation, 24 to >12 h before ovulation, 12 to 0 h before ovulation, or >0 h after ovulation). Recovery rate was calculated by dividing the total number of embryos and UFO by the number of CL. Fertilization rate was calculated by dividing the number of embryos by the number of embryos plus unfertilized oocytes. Data were analyzed by using the General Linear Model procedure of SAS (SAS Institute Inc., Cary, NC). The number of embryos, number of

unfertilized oocytes, fertilization rate, and recovery rate were analyzed using a model that included the effects of treatment (LD, HD, or MD), time (insemination 40 to >24 h, 24 to >12 h, 12 to 0 h, or >0 h relative to ovulation) and treatment by time. Other data (estrus to ovulation interval, estrus to insemination interval, insemination to ovulation interval, time of ovulation relative to duration of estrus and number of CL) were analyzed using a model that included the effect of treatment. Means were separated using the Duncan's Multiple Range Test when a significant treatment effect was detected.

3. Results

As expected, gilts assigned to LD, HD, or MD treatments had similar intervals from estrus to ovulation (33.5 ± 1.6 h), estrus to insemination (22.6 ± 0.6 h), and insemination to ovulation (11.0 ± 1.5 h; Table 1). Likewise, time of ovulation was after 59.5 ± 2.0% of the estrus period and was not affected by treatment.

Ovulation rate was similar for LD, HD, and MD gilts (14.5 ± 0.4 CL per gilt; Table 1). The number of embryos and UFO recovered after uterine flush was also similar (12.3 ± 0.4 embryos and UFO per gilt). The overall embryo and UFO recovery rate [(number of embryos plus UFO)/number of CL] was 85.6 ± 2.6%. The number of embryos recovered ($P < 0.04$), the number of UFO recovered ($P < 0.05$), and the fertilization rate ($P < 0.07$) were affected by treatment. The LD gilts had fewer embryos and more UFO than the MD gilts. The LD gilts also had a lower fertilization rate compared to MD gilts. Although there was a main effect of

Table 1
Characteristics (mean ± S.E.M.) of estrus and ovulation and recovery of embryos and unfertilized oocytes (UFO) from gilts inseminated with either a single low dose (LD; one insemination of 0.5×10^9 spermatozoa), a single high dose (HD; one insemination of 3×10^9 spermatozoa) or multiple high doses (MD; two inseminations of 3×10^9 spermatozoa per insemination) of spermatozoa

	Treatment			
	LD	HD	MD	*P*
Number	33	17	9	
Interval (h)				
Estrus to ovulation	31.1 ± 2.2	35.5 ± 3.0	38.4 ± 4.1	NS
Estrus to insemination	22.5 ± 0.9	21.4 ± 1.2	25.0 ± 1.6	NS
Insemination to ovulation	8.6 ± 2.1	14.1 ± 2.9	13.5 ± 3.9	NS
Estrus duration	54.9 ± 3.2	59.9 ± 4.5	58.5 ± 6.2	NS
Time of ovulation (% of estrus)	58.5 ± 3.0	58.4 ± 4.0	60.0 ± 6.0	NS
Uterine flush				
CL (*n*)	14.4 ± 0.5	14.7 ± 0.7	14.1 ± 0.9	NS
Embryos and UFO (*n*)	12.2 ± 0.6	12.6 ± 0.8	12.0 ± 1.1	NS
Recovery rate (%)	85.5 ± 3.5	85.2 ± 4.8	86.4 ± 6.6	NS
Embryos (*n*)	4.2 ± 1.2[b]	8.1 ± 1.9[a,b]	9.7 ± 2.2[a]	$P < 0.04$
UFO (*n*)	8.5 ± 1.1[a]	5.9 ± 1.8[a,b]	2.5 ± 2.1[b]	$P < 0.05$
Fertilization rate (%)	34.5 ± 8.9[b]	56.5 ± 14.4[a,b]	77.2 ± 16.4[a]	$P < 0.07$

Means and percentages with no superscripts (a, b) in common differ at $P < 0.05$ (Duncan's Multiple Range Test).

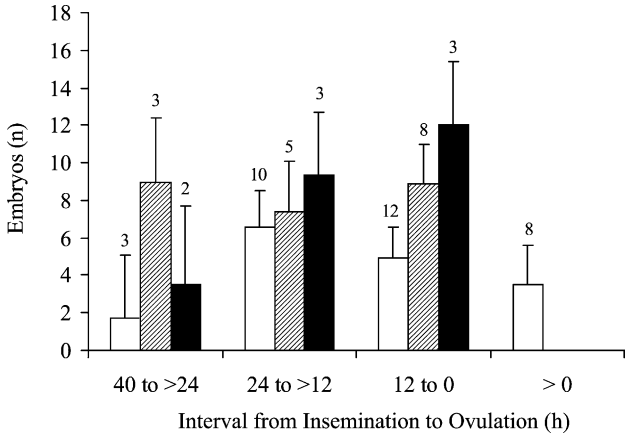

Fig. 1. Least square means and S.E.M. (bar) for the number of embryos recovered at Day 5 after the onset of estrus for gilts inseminated at different intervals relative to ovulation with either a single low dose (LD, □; one insemination of 0.5×10^9 spermatozoa), a single high dose (HD, ▨; one insemination of 3×10^9 spermatozoa) or multiple high doses (MD, ■; two inseminations of 3×10^9 spermatozoa per insemination) of spermatozoa. For gilts given multiple doses, the second insemination was used to determine the interval from insemination to ovulation. There was one gilt in each of the high dose and multiple dose treatments at >0 h and their data are not shown. Number above error bar is the number of gilts contributing to the mean.

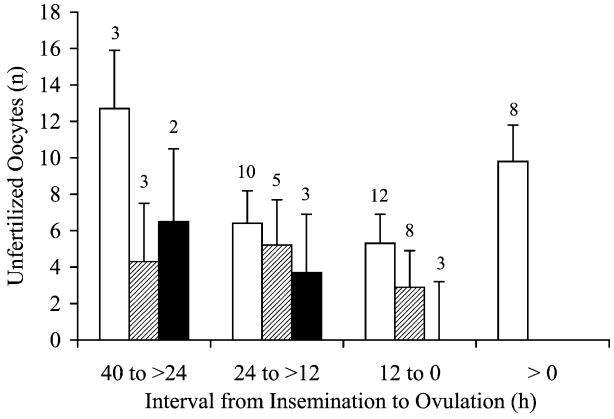

Fig. 2. Least square means and S.E.M. (bar) for number of unfertilized oocytes recovered at Day 5 after the onset of estrus for gilts inseminated at different intervals relative to ovulation with either a single low dose (LD, □; one insemination of 0.5×10^9 spermatozoa), a single high dose (HD, ▨; one insemination of 3×10^9 spermatozoa) or multiple high doses (MD, ■; two inseminations of 3×10^9 spermatozoa per insemination) of spermatozoa. For gilts given multiple doses, the second insemination was used to determine the interval from insemination to ovulation. There was one gilt in each of the high dose and multiple dose treatments at >0 h and their data are not shown. Number above error bar is the number of gilts contributing to the mean.

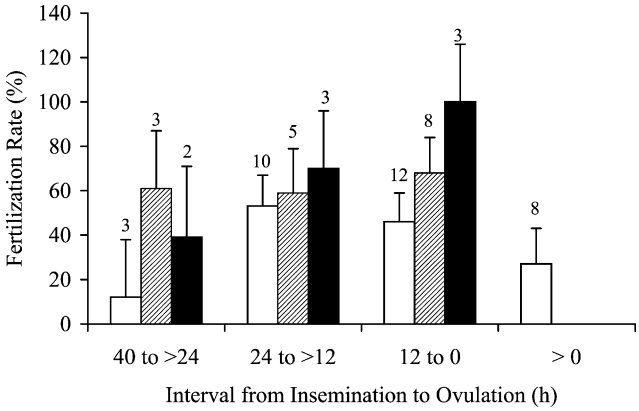

Fig. 3. Least square means and S.E.M. (bar) for fertilization rates for gilts inseminated at different intervals relative to ovulation with either a single low dose (LD, □; one insemination of 0.5×10^9 spermatozoa), a single high dose (HD, ▨; one insemination of 3×10^9 spermatozoa) or multiple high doses (MD, ■; two inseminations of 3×10^9 spermatozoa per insemination) of spermatozoa. For gilts given multiple doses, the second insemination was used to determine the interval from insemination to ovulation. There was one gilt in each of the high dose and multiple dose treatments at >0 h and their data are not shown. Number above error bar is the number of gilts contributing to the mean.

treatment for embryos, UFO, and fertilization rate, the effect of insemination time, and the treatment by insemination time interaction were not significant (Figs. 1–3).

4. Discussion

Gilts inseminated with the LD treatment had a lower fertilization rate than MD gilts. The HD gilts were intermediate between LD and MD. Neither the time of insemination nor the treatment by time of insemination interaction affected fertilization rate. Thus, spermatozoa concentration was the most important factor determining fertility in this experiment. Our data differ slightly from those of Steverink et al. [8] who found only minor nonsignificant effects of sperm dose on fertilization. The present study design differs from that of Steverink et al. [8] because gilts were used instead of sows and a lower sperm dose was tested. Our results suggest either parity affects fertilization results or the lower sperm dose that we used was below the minimum threshold for complete fertilization.

The main effect of time of insemination did not significantly affect fertilization rate or the number of embryos. There was, however, a numeric trend for a greater number of embryos, a higher fertilization rate and fewer unfertilized oocytes when inseminations occurred within 24 h before ovulation (Figs. 1–3). Thus, our data support the original work that showed improved fertilization rates for inseminations given near the time of ovulation [4–7]. We also confirmed previous observations of high variability in fertilization rates across insemination intervals [1]. Some gilts within the present study were inseminated more than 24 h before ovulation but had a 100% fertilization rate. The mechanisms through which individual pigs achieve a high fertilization rate from a poorly timed insemination are not known.

Although the present experiment was one of the largest of its kind, there were a relatively small number of gilts within each treatment by time category (2–12 gilts per category, Figs. 1–3). We also used nine boars. The boar effects were randomized but they may have contributed to the variation in fertility. The small number of gilts within each category and the large number of boars increased the likelihood of a type II statistical error (accepting the null hypothesis when the alternative hypothesis is true) for the treatment by time interaction. Our analyses failed to reject the null hypothesis for treatment by time because there were not adequate grounds for rejection. Failing to reject the null hypothesis does not prove that the null hypothesis is true (i.e. time of insemination relative to ovulation has no effect on fertilization and that insemination dose does not influence this relationship). Perhaps a larger study would have detected a treatment by time interaction and identified specific insemination times that enable the use of lower insemination doses. In the present study, the means for LD gilts inseminated 24 to >12 h before ovulation were nearly identical to the means for HD and MD gilts (Figs. 1–3). Future studies should focus on this time frame and include a larger sample size.

We found that gilts ovulated after approximately 60% of the estrus period had passed (Table 1) and that ovulation was 30–40 h after the onset of estrus. Our data agree with previous findings in gilts [10] and sows [3,4]. In both gilts and sows, therefore, the duration of estrus is a good indicator of the time of ovulation [3,11]. A second method to predict time of ovulation based on ultrasonographic measurement of preovulatory follicle diameter was tested by Soede et al. [12]. They concluded, however, that preovulatory follicle diameter is not an accurate predictor of interval to ovulation. Poor timing of insemination relative to ovulation is believed to be one factor that diminishes reproductive performance on swine farms [3]. More reliable methods for predicting the time of ovulation, however, will need to be identified before inseminations can be administered at a precise time relative to ovulation.

Acceptable fertility has been achieved when lower doses (5×10^6–5×10^8) of sperm were deposited directly into the uterine body of sows and gilts [13–16]. Commercial feasibility of low-dose uterine insemination of sows using 80 ml doses with 1×10^9 spermatozoa has been demonstrated [17]. Uterine insemination, therefore, may enable the use of low sperm doses in gilts as well as sows. The interaction of insemination location (cervix versus uterine body) and insemination time (relative to ovulation) will need to be tested in future experiments.

In conclusion, gilts were inseminated with either low or high doses of spermatozoa around the time of ovulation. A low cervical dose of sperm resulted in unacceptable fertilization rates regardless of time of insemination relative to ovulation. Methods to improve the timing of insemination relative to ovulation, therefore, may not enable the use of cervical insemination with low sperm doses.

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