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Characterization of estrus profile in female swine and its accuracy in estimating ovulation time in comparison to ultrasound diagnosis

M.V.F. Alvarenga, I. Bianchi, E. Schmitt, A.S. Varela Junior, O. Calderam, M.N. Corrêa, J.C. Deschamps, T. Lucia Jr.[1]

PIGPEL, Centro de Biotecnologia, Faculdade de Veterinária, Universidade Federal de Pelotas, 96010-900, Pelotas, RS, Brazil.

Abstract

The objective of this study was to characterize the estrous profile in female swine in order to estimate the moment of ovulation, by determining the weaning-to-estrus interval (WEI) and estrus duration based on conventional estrus detection procedures. In addition, the accuracy of the estimation was evaluated by comparison to ovulation diagnosis using ultrasound. Starting at weaning, 147 females were submitted to back pressure in the presence of a boar three times daily (6:30, 14:30, and 22:30 h) and to an ultrasound examination twice daily (6:30 and 14:30 h). The onset of the estrus was characterized by the first positive response to back pressure, and the end of the estrus was characterized by the first negative response to back pressure. Ovulation was diagnosed using real-time ultrasound. A linear regression model was used to predict the interval from estrus to ovulation considering the variation in the WEI, and a prediction of ovulation time was generated based on the estrus profile (PREDOV). Estrus duration was divided by 3 to allow estimation of the frequency of females ovulating during or outside the final third of estrus. The accuracy of the PREDOV was compared to a standard (ovulation diagnosed through ultrasound). The mean WEI, estrus duration, and interval from onset of estrus to ovulation were 82.6 ± 30.7, 58.3 ± 17.3, and 45.4 ± 14.4 h, respectively. The intervals from the onset of estrus to ovulation and the end of estrus to ovulation did not differ ($P > 0.05$) considering the WEI, but the weaning-to-ovulation interval was increased with a longer WEI ($P < 0.0001$). In comparison to ovulation diagnosed using ultrasound, the sensitivity and specificity of the PREDOV were 74.2% and 40%, respectively, whereas the positive and negative predictive values were 72.1% and 39.5%, respectively. Thus, reproductive performance may be negatively affected if breeding systems are based only on estrus profile because this method lacks accuracy to estimate ovulation time.

Keywords: weaning-to-estrus interval, estrus duration, ovulation, real-time ultrasound, accuracy, swine.

Introduction

Estrus detection is crucial for artificial insemination (AI) programs in swine because inefficient estrus detection can lead to an increased weaning-to-estrus interval (WEI) and return-to-estrus rate if the timing of insemination is not precisely adjusted to ovulation time (Kemp and Soede, 1996). Such a scenario can negatively influence reproductive efficiency of a breeding herd by increasing the number of non-productive days per female (Dial *et al*., 1992). Conventional estrus detection is based on female back pressure and depends on many factors such as daily frequency, boar stimuli, female parity (Soede and Kemp, 1997), and experience of technicians. Although the mentioned procedures are efficient for estrus detection, they cannot identify the onset and the end of the estrus; therefore, estrus duration is usually unknown. By limiting conventional estrus detection procedures to the disappearance of the back pressure response, some studies reported that estrus duration is quite variable, from 48 to 60 h (Weitze *et al*., 1994; Nissen *et al.*, 1997; Lucia *et al.*, 1999). A negative association between WEI and estrus duration has been reported (Weitze *et al*., 1994; Kemp and Soede, 1996); females that have a short WEI will likely have longer estrus duration and vice versa. However, other studies reported that such an association is not strong (Lucia *et al*., 1999; Corrêa *et al*., 2002). The WEI can be longer for primiparous females (Xue *et al*., 1992; Sechin *et al*., 1999) although both WEI and estrus duration were reported to be similar across parities (Corrêa *et al*., 2002). Thus, signs of estrus can follow an irregular pattern in lower-parity females, including manifestations such as shorter estrus duration and less characteristic expression of estrus signs. Such a pattern could occur in females that have reduced backfat at weaning (Tummaruk *et al*., 2001). Therefore, the estrus profile based on conventional procedures commonly used to predict ovulation time is prone to imprecision.

The use of ultrasound to determine ovulation time indicates that, regardless of estrus duration, ovulation typically occurs during the final third of estrus (Weitze *et al*., 1989; 1994). With such knowledge, AI frequency can be adjusted to ovulation time, ideally within a period of 12-28 h before to 4 h after ovulation (Waberski *et al*., 1994; Kemp and Soede, 1996; Nissen *et al*., 1997). Nevertheless, ultrasound-guided ovulation diagnosis is not routinely used on many farms due to the high cost of the equipment and to the need of specialized training for farm staff. As a consequence, conventional estrus detection is still the main decision-

[1]Corresponding author: thomaz@ufpel.edu.br
Phone/Fax: +55 (53) 3275-7350
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making tool used to determine AI protocols at the farm level, especially due to the potential imprecision that has not been quantified yet. This could be accomplished by following an epidemiological approach, considering the conventional estrus profile as a screening test and then comparing its accuracy to a standard (Greiner and Gardner, 2000; Dohoo *et al*., 2003), which in this case would be time of ovulation diagnosed using ultrasound. Thus, by estimating the accuracy of the estrus profile, it would be possible to determine whether the ovulation time estimated by the estrus profile actually occurred within or outside the final third of estrus. The objectives of this study were to characterize the estrus profile of female swine kept in commercial farm conditions, based on conventional estrus detection and to determine its accuracy in estimating ovulation time compared to ultrasound-determined diagnosis of ovulation.

Materials and Methods

This study was conducted on a commercial farm with a female inventory of 2,250 and located in the northwest of Rio Grande do Sul, Brazil. Within each weekly weaning group (n = 75), half of the females were randomly selected to take part in the experiment. After five weeks, considering the exclusion of females that did not show signs of estrus up to 10 d post weaning and those that were culled, the experiment included 147 F1, crossbred (Landrace X Large White) females ranging in parity from 1 to 8 and from the same genetics. The weaned females were housed in individual crates. Their backfat was evaluated at P2, between the last two ribs, 7 cm to the right and left from the midline, using an ultrasound scanner equipped with a 5.0 Mhz convex-array probe (Anser Vet 485, Pie Medical®) and employing a transcutaneous scanning technique. Feeding regimes during both lactation and gestation followed the recommendations of the National Research Council (1998). The determination of the onset and end of estrus started immediately after weaning and was based on estrus detection via back pressure in the presence of a boar three times daily (6:30, 14:30, and 22:30 h). The onset of estrus was characterized by the first positive response to back pressure minus 4 h (half of the interval between observations), whereas the end of the estrus was characterized by first negative response to back pressure minus 4 h. Thus, estrus duration was estimated by the difference between the onset and the end of the estrus (Weitze *et al*., 1994; Corrêa *et al*., 2002).

All females were subjected to ultrasound evaluation of ovarian condition using the equipment and procedure described above. The exams were conducted at times adjusted to the routine farm management (6:30 and 14:30 h) because farm staff was used for help with this procedure. The females were examined while standing by an experienced technician with the probe positioned on the right side, near to the midpoint between the femur-tibia joint and the last rib, 10 cm above the udder (Weitze *et al*., 1989; 1994).

The experiment was double blinded, meaning that two different teams of technicians were in charge of the ultrasound-determined ovulation diagnosis and estrus detection. Neither team had knowledge of the results obtained by the other team. The occurrence of ovulation was determined when no pre-ovulatory follicles were found on the ovaries or when the follicle number was lower than that observed in the previous exam, as long as this diagnosis was confirmed in the following exam (Soede *et al*., 1994; 1995). After these procedures, the intervals from weaning to ovulation, the onset of estrus to ovulation, and the end of estrus to ovulation were calculated.

Descriptive statistics were calculated for WEI, estrus duration, and the following intervals: weaning to ovulation, onset of estrus to ovulation, and end of estrus to ovulation. Frequency distributions were generated for all potential independent variables. Such variables were categorized according to the dispersion of such distributions as: WEI, < 72, 72-96, and > 96 h; estrus duration, < 50, 50-74, and > 78 h; parity, 1, 2, 3-5, and 6+; and backfat, < 13, 13-15, and > 15 mm.

Analysis of variance (ANOVA) was used to evaluate the effects of WEI, estrus duration, parity, and backfat on the intervals from weaning to ovulation, onset of estrus to ovulation, and end of estrus to ovulation. All comparisons of means were done using the least-significant difference method. Lactation length was tested as an independent variable in all ANOVA models but was excluded from further analysis because it was not significant.

A linear regression model was used to predict estrus duration using the WEI as the independent variable. Another linear regression model was used to predict the interval from the onset of estrus to ovulation using the WEI as the independent variable. The value of the WEI observed for each female was subsequently included in the equation generated by the second regression model mentioned above. The resulting value was defined as the predicted ovulation time based on the estrus profile (PREDOV).

Estrus duration observed for each female was divided by three, based on the assumption that swine females typically ovulate during the final third part of estrus (Weitze *et al*., 1994; Soede *et al*., 1994; 1995; Nissen *et al*., 1997). The time of both PREDOV and ovulation diagnosed by ultrasound was categorized according to its occurrence either during or outside of the final third of estrus. The frequencies of ovulation estimates according to both methods were cross-tabulated and measures of accuracy (sensitivity, specificity, and positive and negative predictive values) for the PREDOV were calculated as described elsewhere (Greiner and Gardner, 2000; Dohoo *et al*., 2003), considering the ultrasound-determined diagnosis of ovulation as the standard. All statistical analyses were conducted with the SAS® software (SAS, 1999).

Results

The mean WEI was 82.6 ± 30.7 h, and the mean estrus duration was 58.3 ± 17.3 h. The mean intervals of weaning to ovulation, onset of estrus to ovulation, and end of estrus to ovulation were 131.6 ± 32.3, 45.4 ± 14.4, and 12.3 ± 14.4 h, respectively. Mean lactation length was 19.5 ± 2.0 d.

Although the WEI did not influence (P > 0.05) the intervals from onset of estrus to ovulation and end of the estrus to ovulation (Table 1), the weaning-to-ovulation interval was prolonged with longer WEI (P < 0.0001). The variation in estrus duration as a function of the WEI is expressed by the following linear regression model: estrus duration = 72.6717 – 0.17404 (WEI), R2 = 0.0954 (P < 0.0001).

Table 1. Intervals from onset of estrus to ovulation, end of estrus to ovulation, and weaning to ovulation as a function of the weaning-to-estrus interval (WEI).

WEI (h)	n	Weaning to ovulation (h)	Onset of estrus to ovulation (h)	End of estrus to ovulation (h)
< 72	34	89.9 ± 3.7[a]	48.1 ± 2.3	14.2 ± 2.3
72 – 96	63	138.0 ± 3.0[b]	46.4 ± 1.9	15.4 ± 1.9
> 96	50	157.0 ± 3.2[c]	43.2 ± 2.1	12.6 ± 2.0
Overall	147	131.3	45.4	12.3

[a,b,c] Means ± SEM with different superscripts within columns differ (P < 0.05).

The weaning-to-ovulation interval did not differ (P > 0.05) across the categories based on estrus duration (Table 2). However, the interval from the onset of estrus to ovulation was generally prolonged with longer estrus duration (P < 0.05), and an estrus duration longer than 74 h was associated with longer interval from the end of estrus to ovulation (P < 0.0001) compared to the intervals observed with a shorter estrus duration. Both the intervals from the onset of estrus to ovulation and the end of estrus to ovulation and the weaning to ovulation interval were not influenced (P > 0.05) by either parity (Table 3) or backfat (Table 4). In all ANOVA models, no significant interaction among independent variables was observed.

Table 2. Intervals from onset of estrus to ovulation, end of estrus to ovulation, and weaning to ovulation as a function of estrus duration (ED).

ED (h)	n	Weaning to ovulation (h)	Onset of estrus to ovulation (h)	End of estrus to ovulation (h)
< 50	39	126.5 ± 3.6	38.2 ± 2.3[a]	6.5 ± 2.3[d]
50 – 74	81	125.4 ± 2.5	46.9 ± 1.6[b]	10.4 ± 1.6[d]
> 74	27	132.8 ± 4.1	52.6 ± 2.6[c]	25.3 ± 2.6[e]

[a,b,c] Means ± SEM with different superscripts within columns differ (P < 0.05).
[d, e] Means ± SEM with different superscripts within columns differ (P < 0.0001).

Table 3. Intervals from onset of estrus to ovulation, end of estrus to ovulation, and weaning to ovulation as a function of parity.

Parity	n	Weaning to ovulation (h)	Onset of estrus to ovulation (h)	End of estrus to ovulation (h)
1	28	133.7 ± 4.1	47.6 ± 2.7	13.2 ± 2.6
2	34	130.5 ± 3.7	42.7 ± 2.4	14.6 ± 2.4
3-5	43	121.1 ± 3.4	45.0 ± 2.2	13.1 ± 2.1
6 +	42	127.6 ± 3.4	48.3 ± 2.2	15.4 ± 2.1

Means ± SEM do not differ (P > 0.05).

Table 4. Intervals from onset of estrus to ovulation, end of estrus to ovulation, and weaning to ovulation as a function of backfat.

Backfat (mm)	n	Weaning to ovulation (h)	Onset of estrus to ovulation (h)	End of estrus to ovulation (h)
< 13	54	126.7 ± 3.0	45.8 ± 1.9	14.5 ± 1.9
13-15	47	124.5 ± 3.2	42.9 ± 2.1	15.2 ± 2.0
> 15	46	133.6 ± 3.2	49.0 ± 2.1	12.4 ± 2.0

Means ± SEM do not differ (P > 0.05).

The PREDOV was estimated by the following regression model: PREDOV = 55.0654 – 0.11663(WEI), R^2= 0.0633 (P < 0.0021). The distributions of both the interval from the onset of estrus to ovulation and PREDOV are shown in Fig. 1. The mean PREDOV was 45.3 ± 3.6 h.

Out of a total of 147 females, ultrasound-determined ovulation diagnosis identified 101 ovulations occurring during the final third of estrus (Table 5). On the other hand, when the estrus profile was used to estimate ovulation time, 104 females would have been estimated as having ovulating during the final third of estrus. However, among those 104 females, only 75 actually ovulated during the final third of estrus (the true positive diagnosis), along with another 26 females that were estimated as having ovulated outside of the final third of estrus according to the estrus profile (false-negative diagnosis). Additionally, 17 ovulations that occurred outside of the final third of estrus corresponded to actual negative diagnoses whereas 29 ovulations corresponded to false-positive diagnoses. Therefore, when compared to ovulation diagnosis by ultrasound, the PREDOV had a sensitivity of 74.2% and a specificity of 40%. The positive predictive value was 72.1% whereas the negative predictive value was 39.5%.

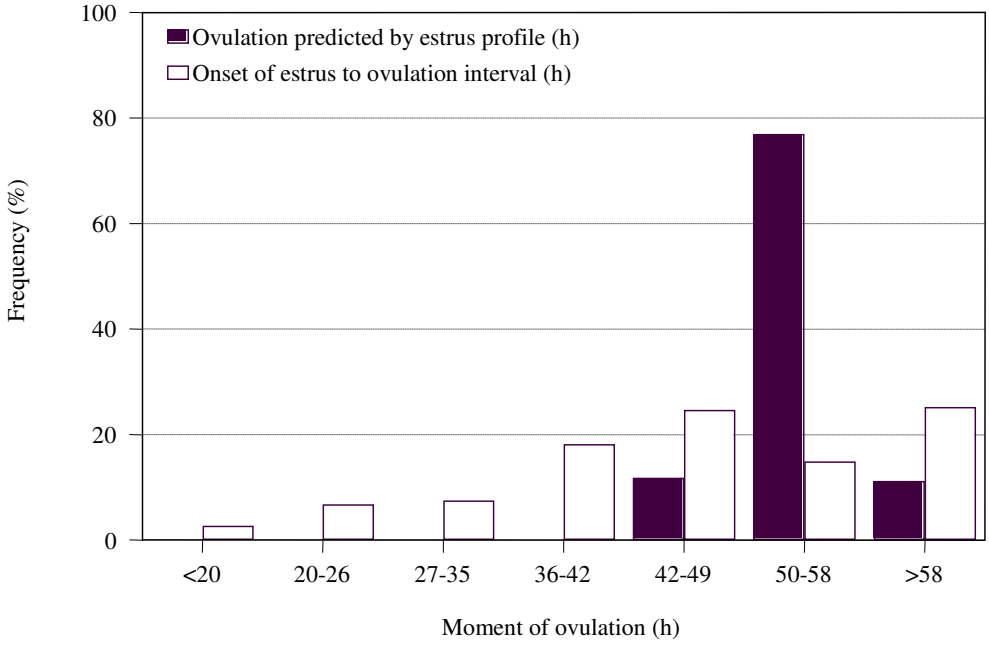

Figure 1. Frequency distribution for the onset of estrus to ovulation interval and the ovulation predicted by estrus profile.

Table 5. Accuracy of ovulation time predicted by estrus profile (PREDOV) in comparison to ovulation time diagnosed by ultrasound, comparing occurrence during or outside of the final third of estrus (total n = 147).

PREDOV	Ovulation diagnosed by ultrasound	
	During the final third of estrus	Outside the final third of estrus
During the final third of estrus	75	29
Outside the final third of estrus	26	17

Sensitivity = 75/(75 + 26) = 0.7423; Specificity = 17/(17 + 29) = 0.3996; Positive predictive value: 75/(75 + 29) = 0.7211; Negative predictive value: 17/(26 + 17) = 0.3953.

Discussion

Compared to ultrasound-determined diagnosis of ovulation, the estrus profile had limited accuracy in estimating ovulation time. Its moderate sensitivity indicates that only 74% of the ovulations that occurred during the final third of estrus would have been correctly estimated by conventional estrus detection procedures. The false-negative estimates of ovulation time based on estrus profile would be too early when compared to the actual time of ovulation, which could lead to losses in reproductive efficiency characterized by a higher return-to-estrus rate, lower farrowing rate, and increase in non-productive days (Dial *et al.*, 1992). However, such potential losses may be minimized by the use of multiple artificial inseminations (AI) during estrus, assuming that at least one of those AIs would result in semen deposition in the female genital tract close to the time of ovulation (Corrêa *et al.*, 2002). This fact, along with the high cost of the equipment and the

need for skilled staff to implement ultrasound diagnosis, may justify why conventional estrus detection is still the method of choice for AI protocols at most farms.

The poor specificity of the PREDOV indicates that, among the ovulations that occurred outside of the final third of estrus, only 40% of them (the true negative diagnosis) would be accurately estimated through conventional estrus detection. For most of the false-positive estimates of ovulation time based on estrus profile, the use of multiple AIs would prevent substantial losses in reproductive efficiency. However, if time of ovulation was estimated to have occurred after the actual time of ovulation, there would be a higher probability of performing post-ovulatory inseminations, which have been associated with a reduction in farrowing rate and litter size due to post-breeding inflammatory processes in the uterine lumen and embryonic losses due to leukocyte influx (Rozeboom *et al.*, 1997; 1999; Kaeoket *et al*., 2005). Additionally, conventional estrus detection had moderate positive predictive value and limited negative predictive value.

Over the years, accuracy has been used to evaluate the efficiency of diagnostic methods focused on disease monitoring and control (Greiner and Gardner, 2000; Dohoo *et al*., 2003), but such concepts have not been thoroughly explored in other areas. In studies that aimed to diagnose pregnancy, rates of non-return to estrus estimated through conventional estrus detection procedures were described to be more accurate than ultrasound diagnosis (Almond and Dial, 1986a; b). Such studies evaluated less sophisticated ultrasound devices, whose results may be confounded depending on the bladder's content. Such findings were contradicted by a study reporting that ultrasound diagnosis would be more precise than the pregnancy diagnosis based on rate of non-return to estrus (Viana *et al*., 2002).

The distributions in Fig. 1 indicate that despite the fact that the onset of estrus-to-ovulation interval and the PREDOV had similar means, PREDOV was concentrated within 42-58 h post-estrus detection whereas true ovulation time had a wider dispersion, as confirmed by other studies (Kemp and Soede, 1996; Belstra *et al*., 2004), with more than 10% of the ovulations occurring before 36 h after estrus detection and more than 20% of them occurring after 58 h post-estrus detection. The estrus profile was imprecise in estimating ovulation time despite the fact that it was based on three daily estrus detections, which is unlikely to be routinely used in farm management due to increased labor costs. Actually, on the farm where the study was conducted, estrus detection was routinely conducted twice daily, which could increase the risk of subsequent reproductive failure if estrus detection is not conducted properly.

The associations among WEI, estrus duration, and ovulation time reported in this study are generally consistent with the literature (Weitze *et al*., 1994; Kemp and Soede, 1996; Nissen *et al*., 1997), but the reduced coefficients of determination observed for the linear regression models in this study emphasize that not only the association between WEI and estrus duration is weak, as reported elsewhere (Lucia *et al*. 1999; Corrêa *et al*., 2002), but also estimates of ovulation time as a function of the WEI are weak as well. It is important to consider that such an association can vary according to farm-specific factors (Viana *et al.*, 2002; Belstra *et al*., 2004). The intervals from the onset of estrus to ovulation and end of estrus to ovulation were not associated with the WEI but were obviously associated with estrus duration as reported in other studies (Waberski *et al*., 1994; Viana *et al*., 1999; 2002). The findings of this study give additional support to the assumption that AI programs based only on the WEI may be prone to inefficiency (Viana *et al*., 1999; Corrêa *et al*., 2002). Our data suggest that, even when using conventional estrus detection and multiple AIs, AI protocols would be more efficient if the first AI is conducted nearly 24 h after the estrus detection and followed by further AIs at 12-h intervals because most females began to ovulate after 42 h post-estrus detection. It is important to point out that the double-blinded design used in this study guaranteed that the results observed during the estrus profile were not influenced by the previous knowledge of the ultrasound-determined ovulation diagnosis and vice versa. Additionally, the estrus profile was not influenced by female parity and backfat, indicating that even considering the studies that reported longer WEI for primiparous females (Xue *et al*., 1992; Sechin *et al*., 1999) or for those having lower backfat (Tummaruk *et al*., 2001), such effects would not be associated with irregular estrus profiles.

In this study, ultrasound-determined diagnosis of ovulation was conducted using a transcutaneous technique. Even though the trans rectal exam requires less-specific training, the transcutaneous technique would provide similar results (Nissen *et al*., 1997). Furthermore, it is possible that conducting ultrasound exams within intervals shorter than those used in the present study (Kemp and Soede, 1996; Belstra *et al*., 2004) may improve the accuracy in detecting ovulation time. If so, the definition of the standard would be different from that used in this study. However, in the present study, the use of ultrasound exams at shorter intervals was limited due to labor constraints associated with the routine management practiced on that farm, which probably represents the management conditions of many commercial farms. Under such circumstances, conducting ultrasound exams at shorter intervals or during the night, as occurred with the estrus detection in the present experiment, could only be accomplished by breaking the double-blinded design of the experiment. Additionally, the ovulation time observed in this study was consistent with data observed with shorter diagnosis intervals (Kemp and Soede, 1996). Therefore, the reported estimates appropriately reflect the management conditions of the commercial farm where the present study was conducted.

In conclusion, the estrus profile is a screening test with limited accuracy in estimating ovulation time when compared to the ultrasound-determined diagnosis

of ovulation. However, the routine use of ultrasound on commercial farms is still limited by the high cost of the equipment and the need of specially-trained farm staff. Therefore, most farms rely on conventional estrus detection to establish AI protocols and use multiple AIs per estrus.

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