Application of modern estrus detection protocols in small scale Hybrid Black pig production systems

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Abstract. The aim of this study was to re-invent the readily available estrus detection methods in small scale with black colour pigs, capable to yield more accurate results. Data were collected from 32 Meishan X Large Black X Duroc Hybrid Black sows housed in groups. Weaned sows were observed for 11 days, and estrus was detected twice daily, comprising of live observations of mating behaviour, a boar test and a back pressure test. The findings revealed a strong inverse relationship (r = -0.885) between time spent on lying and standing, which could be used as predictive marker for approaching estrus (p ≤ 0.05). The mating-related behaviours were found to be significantly higher during 5th to 7th day post weaning. There were strong positive correlations between nosing genital and bar-biting (r = 0.809), mounting and voice change (r = 0.743), and voice change and nosing genitals (r = 0.731). Sow body temperature varied during estrus, with vulva and vulva skin temperatures providing a better fit to the data, R^2 = 0.455 and R^2 = 0.667 for summer and winter trials, respectively. The findings revealed that with or without a boar, when on heat, sows would respond positive to back pressure test. In conclusion, behavioural and physiological changes of the vulva provided an opportunity for primary diagnosis of estrus. The flexibility of the infrared thermometer to be combined with other estrus detection protocols yielded more accurate results, providing the small scale farmers with an opportunity to apply modern detection protocols.

Keywords: Hybrid Black sow, estrus detection, behaviour, infrared thermometer.

INTRODUCTION

Accurate estrus detection has been shown to be a critical determinant for increased profitability and production efficiency within the swine industry (Seo *et al.*, 2012; Andersson *et al.*, 2016; Johnson and Shade, 2017). Extensive research work has enabled farmers to plan their insemination in such a way that it is performed within 12 to 24 hours before ovulation, where fecundation rates are maximum (Soede *et al.*, 1995; Nissen *et al.*, 1997; Knox *et al.*, 2017). Unfortunately, until now, there are no protocols developed to accurately predict when ovulation will occur in sows, not even the ultra-sound guided ovulation diagnosis is routinely used in a farm setting due to its high costs and need for special skills (Alvarenga *et al.*, 2006). At the present time, we rely on detection of standing heat as the reference point to predict the best time to inseminate for maximum fertilization rate (Knox, 2016; Knox *et al.*, 2017; Knox *et al.*, 2018).

However, estrus detection is very difficult and remains a challenge to most swine breeders. And with the increased number of pigs per farm, the conventional estrus detection methods have been rendered impractical

and inefficient to use (Seo *et al.*, 2012; Andersson *et al.*, 2016; Johnson and Shade, 2017). Erratic estrus detection results to a significant financial loss to the farmers (Andersson *et al.*, 2016). A missed insemination opportunity translates to additional 21 days of feeding an unproductive sow, increased number of non-productive days and reduced number of piglets harvested per sows per year (King *et al.*, 1998; Andersson *et al.*, 2016). Consequently, drugs to induce ovulation (Knox, 2015; Andersson *et al.*, 2016) and other high-tech estrus detection protocols such as sensors, pedometers, pudendal images and ultrasound have since been introduced with an aim of replacing the traditional labour intensive and unreliable estrus detection techniques.

Two to three days before onset of estrus, increasing estrogen levels from developing ovarian follicles stimulate increased blood flow and fluid retention in the reproductive tract and cause the vulva and clitoris to swell and turn red. This is commonly observed in gilts, but it is usually only seen in sows if the vulva is parted to examine the interior. However, Hybrid Black gilts and sows both have tended to have dark vulva colour. The characteristics of the pig's body surface colour affect the observation of estrus, especially if the so-called vulva turning red is difficult to identify from Hybrid Black pigs.

However, we must realize that the applicability of such estrus detection protocols in some parts of the world may be limited, particularity in the developing world where production is mainly dominated by small scale producers, due to lack of financial resources and inavailability of skilled labour (Simões *et al.*, 2014; Knox, 2015). Furthermore, the utilization of hormones might be dependent on the regulatory approval in that particular country. At present time, small scale production systems still rely on the widely criticized error prone behaviour-based traditional estrus detection methods due to financial constraints.

Smallholder farmers have been identified as crucial role players in ensuring food security (Herrero *et al.*, 2010). Therefore, whether one is engaged in small scale production with less than 10 pigs or in large scale production with more than 300 sows, the need for efficient estrus detection methods remains the same. And as researchers, we have the responsibility to develop simple and affordable but more accurate estrus detection protocols. To achieve this, we must build from existing literature and investigate the most appropriate way of crafting observed sow behavioural changes with the other traditional methods of estrus detection.

Previous studies have reported vulva mucus discharge, reddening and swelling as the mostly used physical signs for estrus detection (Seo *et al.*, 2012; Johnson and Shade, 2017). Another notable contribution from literature is the reported increase in physical activity on the onset of estrus (Freson *et al.*, 1998; Pedersen *et al.*, 2003; Cornou, 2006; Pedersen, 2007). When on estrus, sows

will perform mating-related behaviours such as mounting and standing to be mounted, boar seeking, vocalization, ano-genital sniffing, flank nosing and bar biting (Pedersen *et al.*, 2003; Cornou, 2006; Pedersen, 2007; Johnson and Shade, 2017; Miura *et al.*, 2017). Mounting and allowing to be mounted have been by far the most used proceptive behaviour to detect estrus (Pedersen *et al.*, 2003; Pedersen, 2007; López-Gatius, 2012; Seo *et al.*, 2012; Shahinfar *et al.*, 2014; Johnson and Shade, 2017).

In the present study, we seek to demonstrate how smart integration of the boar test, back pressure test, observed vulva and sow behavioural changes together with a temperature variation measured at six anatomical body sites can yield a simple but more accurate estrus detection protocol suitable for small scale Hybrid Black pig production systems. This assertion is based on the hypothesis that when sows approach estrus, vulva swelling, reddening and mucus discharge are observed, accompanied by increased sow activity and performance of mating-related behaviours. Moreover, when exposed to a boar test or subjected to back pressure test, sows on estrus will stand rigidly and remain immobile (Alvarenga *et al.*, 2006; Knox *et al.*, 2017). Specifically, in this study we aim to re-invent the readily available estrus detection methods to one multi-parameter approach which is capable to yield more accurate results through optimized management among small scale Hybrid Black pig production farmers.

MATERIALS AND METHODS

Animals and housing

This study took place in a private commercial pig breeding farm located in the outskirts of Pingtung city in Taiwan. The experimental animals comprised of 32 Meishan × Large Black × Duroc cross bred multiparous sows housed in group pens measuring 5.2 m in length and 5.2 m in width with an average temperature of 28.2 ± 2.2°C. Summer data was collected from July to August 2018 and winter data was from January to February of the following year. Realizing that one of the parameters studied was behaviour, group pens were preferred for their appropriateness to allow sows to express their normal behaviours.

Behavioural observations

Straight from weaning, sows were marked with identification numbers using a spray paint and observed until the 11th day post weaning. Behaviours were scored by two observers during live observations using 1-minute instantaneous scan sampling. A total of four hours of observations per day were carried out 1 hour before and

after morning feeding (7:00 to 9:00 A.M.) and 1 hour before and after afternoon feeding (2:00 to 4:00 P.M.). The procedure resulted into 240 scans per day per pig (4 hours × 60 scans). In the present study, non-specific information on sow general behaviour and physical activity was collected to generate a reliable baseline upon which we could relate, to easily recognise the behavioural changes due to the onset of standing heat. The idea was to gain an insight on behavioural mechanism underlying standing heat. The following general behaviours were observed during 11 consecutive days: lying, standing, drinking, urinating, defecating, eating, and fighting (aggression).

Estrus detection

Estrus detection was performed twice, daily (morning at 9:00 A.M. and afternoon at 4:00 P.M.), by the two observers under the guidance of the farm manager, who has more than 22 years of experience in swine breeding and management. Physiological changes of the vulva and appearance of mating-related behaviours served as screening sieves. Sows were then subjected to the boar test, and this was achieved by walking a four-year old sexually matured Duroc boar, with observers focusing on the response shown by sows suspected to be on estrus. Sows recognized to be exhibiting the standing reflex and lordosis were considered to be on estrus. To further confirm the claim, identified sows were then subjected to a back pressure test. Sows that stood rigid and remain locked to back pressure, with flapping or erect ears reflex, without vocalization were confirmed to be on standing heat. On the other hand, those who vocalized, moved around and escaped back pressure, were considered to have not yet reached standing heat. The first negative response to back pressure marked the end of standing heat.

Body temperature detection

To evaluate temperature variation in relation to estrus, the body temperature of the animals was measured from six anatomical body sites: the vulvar skin; intra-vulvar skin; gluteal area; udder; ears; and the back. A hand held infrared digital thermometer (FUNET EM-350B, E-News Hardward Co., Ltd. Taipei, Taiwan) was used to measure body temperature. The thermometer was held at 10 cm from the skin of the measure anatomical body site of the sow, with 90° incidence and remained fixed in the measure area for a period of 10 seconds to ensure that an absolute temperature was recorded.

The strategy was to integrate an infrared thermometer with multiple heat detection methods to screen-off false alarms from group-mixing for more accurate results, thereby reducing the reliance on boars. In this study, we

hypothesized that the pro-estrus phase will be marked by an increased sow activity together with an increase in body temperature, and then followed by a decrease in body temperature and disappearance of proceptive behaviours among sows.

Statistical analysis

The observed behaviours were summed up and their averages were expressed as a proportion of the total observation time and entered in Microsoft Excel database. Transformed data was then statistically analyzed using SPSS version 23.0, by performing one-way analysis of variance (ANOVA). Means were compared according to Fisher's least significance difference (LSD) procedure. Pearson linear correlations were also used to examine the relationship between the observed behaviours. Each sow was considered as an experimental unit and means were considered statistically significant at $p \leq 0.05$.

RESULTS AND DISCUSSION

Currently, we rely on the onset of standing heat as a point of reference to predict ovulation time, to inseminate when fertility rate is at maximum (Knox et al., 2017; Knox et al., 2018). Therefore, improved precision on estrus detection is premium for high reproductive performance. Herein, the present study aimed to strategicaly combine the following traditional estrus detection methods to yield more accurate results: behavioural observations; physical and physiological changes of the vulva; the boar test; and the back pressure test. Procedures performed in this study are in line with suggestions from previous studies that advocated for combination of multiple estrus detection protocols for more accurate results (Geers et al., 1997; Firk et al., 2002; Cornou, 2006; Luño et al., 2013).

Previous research studies reported that normally pigs take 4 to 7 days to return to estrus (Kemp and Soede, 1996; Soede et al., 2011; Knox, 2015; Kraeling and Webel, 2015). However, results in the present study indicated a considerable variation, reporting a 7 to 10 days weaning-to-estrus interval (WEI). Probably, the long WEI could be due to genetic influence as the study used Meishan crossed sows, a native breed known for a large litter size. Perhaps, feed intake during lactation, length of lactation, litter size, suckling intensity of piglets might have translated the poor body condition score at weaning, thus the sows needed more time to recover.

Another probable factor is seasonality, as the study was conducted during the brutally hot temperatures of summer season (that is, 28.9 ± 2.2°C). The high ambient temperatures might have negatively impacted on the reproductive performance of weaning sows, thus
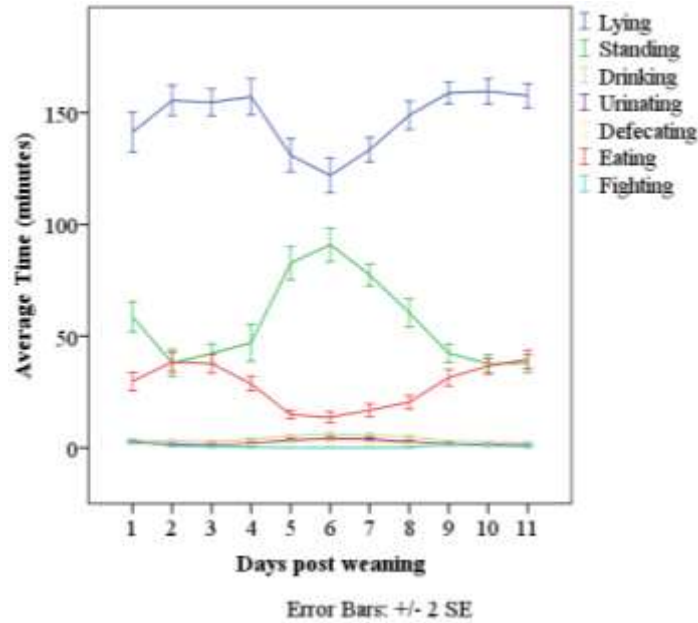
Figure 1. Variation in time duration of all the activities performed by sows according to the day of experiment, from weaning to post estrus (n = 32). **Note** that the total time of observation was 240 minutes per day.

Table 1. Mean variation in time duration of all the activities performed by sows according to the day of experiment, from weaning to post estrus, summer trial (n = 32).

	Lying	Standing	Drinking	Urinating	Defecating	Eating	Fighting
1st day	141.38[bcd]	58.63[b]	3.13[cd]	2.42[bcd]	1.71[cd]	29.71[c]	3.04[a]
2nd day	155.67[ab]	38.04[c]	3.04[cd]	1.54[de]	1.00[de]	38.42[ab]	1.00[bcd]
3rd day	154.67[ab]	42.25[c]	2.71[cd]	1.33[de]	0.83[e]	37.71[ab]	1.08[bc]
4th day	157.21[a]	47.08[bc]	3.88[bc]	1.96[cde]	1.42[cd]	28.67[c]	0.33[cde]
5th day	131.04[de]	82.67[a]	5.33[a]	3.46[ab]	2.63[ab]	14.83[d]	0.04[e]
6th day	121.88[e]	90.96[a]	6.17[a]	4.08[a]	3.13[a]	13.71[d]	0.08[e]
7th day	133.50[cde]	77.42[a]	5.75[a]	3.88[a]	2.54[ab]	16.83[d]	0.08[e]
8th day	148.92[abc]	60.50[b]	4.88[ab]	2.71[bc]	1.96[bc]	20.42[d]	0.21[de]
9th day	158.83[a]	42.33[c]	3.00[cd]	1.71[cde]	1.25[cde]	31.46[bc]	1.42[b]
10th day	159.58[a]	37.67[c]	2.50[cd]	1.50[de]	1.04[de]	36.63[ab]	1.08[bc]
11th day	157.71[a]	37.83[c]	2.38[d]	1.25[e]	0.83[e]	39.58[a]	0.46[cde]

[a, b, c, d,e]Means with different superscripts within columns are statistically different from each other (p ≤ 0.05). **Note** that the total time of observation was 240 min per day.

resulting in the long WEI, as reported in other studies (Messias De Bragança *et al.*, 1998; Quiniou and Noblet, 1999; Mayorga *et al.*, 2019). Furthermore, multiparous sows were used in the current study, and parity might have had influenced on the WEI. Besides the aforementioned factors, photo-period, social stimulation and the presence of mycotoxins in the feed were also reported to have a potential to influence the WEI (Weitze *et al.*, 1994; Quiniou and Noblet, 1999; Tummaruk *et al.*, 2010; Soede *et al.*, 2011).

Weaning sow general behavioural patterns observed before and after the onset of standing heat

General behaviours performed by sows according to the day of experiment from weaning to post estrus are presented in Figure 1, and a summary of their average time duration is represented in Table 1. Regarding the general behavioural patterns, the major findings of the study revolved around the time spent by sows, either lying or standing. As expected, the time spent on lying by
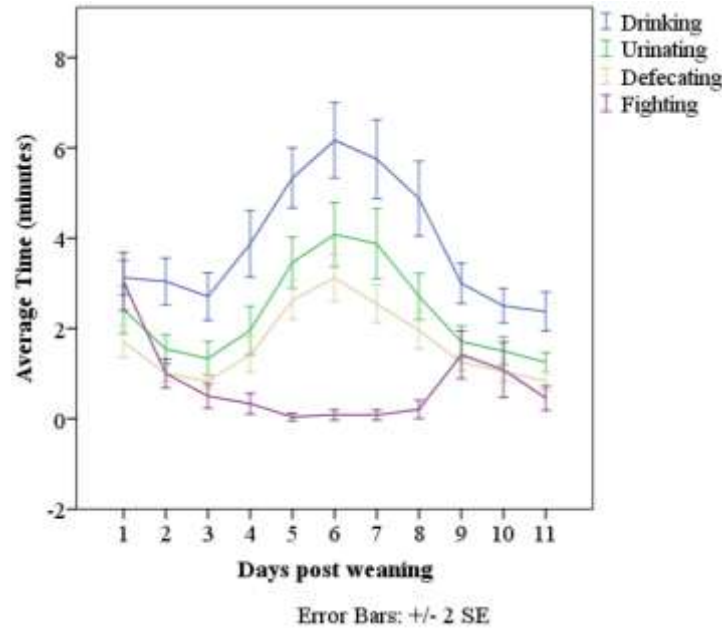
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Figure 2. Variation in time duration of the least observed behaviours performed by sows according to the day of experiment, from weaning to post estrus (n = 32). **Note** that the total time of observation was 240 minutes per day.

sows was significantly higher (p ≤ 0.05) outside the estrus phase (days 1 to 4 and 8 to 11), approximately 155 min of the total 240 min observed per day as shown in Table 1 and Figure 1. In pigs, lying is a normal behaviour as literature states that older pigs can be lying down for up to 90% of their daily time (Ekkel *et al.*, 2003; Spoolder *et al.*, 2012; Nasirahmadi *et al.*, 2015). However, as sows neared the onset of standing heat (day 5), the time spent on lying dropped significantly (p ≤ 0.05), reaching the minimum time at the 6th day post weaning.

In contrast, the time spent standing increased remarkably during the estrus phase (days 5 to 7), with maximum time obtained at the 6th day post weaning, as shown in Figure 1. Increased time on standing marked the beginning of increased physical activity among weaning sows, and the increased likelihood to perform other behaviours. Andersson *et al.* (2016) reported that increased physical activity among farm animals during the estrus phase has since prompted the invention of odometers and accelerometer-based automated estrus detection systems, to deal with the higher numbers in commercial settings.

Another noteworthy behaviour was the significant decrease on time spent on feeding when sows approached estrus phase (days 5 to 7), then followed by a slight increase at post estrus. The findings are in line with several studies which also reported a slights decrease in feed intake during estrus (Friend, 1973;

Walton and King, 1986; Trout *et al.*, 1998; Cornou, 2006). Cornou (2006) further suggested that the decreased feed intake was a result of high levels of oestrogen in the blood. However, other previous studies reported a large variability on feed intake shown by sows during estrus, thus this behaviour could not be used as a determinant of estrus in automated estrus detection protocols.

As shown in Figure 1, aggression, drinking, urinating and defecating were the least observed behaviours in the present study. To gain a clear insight, these behaviours were then shown in an enlarged version of the part correspondent to them in Figure 1, presented as Figure 2.

As reported earlier, increased physical activity towards estrus also increased the likelihood of the occurrence of the least observed behaviours such as drinking, urinating and defecating presented in Figure 2. It is worth noting that these activities were short-lasting, suggesting that probably they might be stereotypic behaviours, perhaps motivated by the anxiety of boar seeking (Shearer and Katz, 2006). Perhaps, frequent urination and defecation were reflex behaviours induced by the presumably high levels of oestrogen in the blood, and resultantly, sows experienced frequent constriction and relaxation of the anal-vaginal muscles. Other than the high ambient temperatures, frequent drinking might have been associated with increased restlessness, and the desire to play with the nipple in drinkers, thus a stereotypic behaviour (Meese and Ewbank, 1973; Bauer and Hoy,

Table 2. Pairwise correlations coefficients (r) between time duration on all observed activities performed by sows during the time of the experiment (n = 32).

Variable 1	Variable 2	r	Probability
Standing	Lying	-0.885	< 0.000*
Drinking	Lying	-0.384	< 0.000*
Drinking	Standing	0.483	< 0.000*
Urinating	Lying	-0.410	< 0.000*
Urinating	Standing	0.499	< 0.000*
Urinating	Drinking	0.728	< 0.000*
Defecating	Lying	-0.404	< 0.000*
Defecating	Standing	0.510	< 0.000*
Defecating	Drinking	0.589	< 0.000*
Defecating	Urinating	0.719	< 0.000*
Eating	Lying	0.178	0.004*
Eating	Standing	-0.586	< 0.000*
Eating	Drinking	-0.581	< 0.000*
Eating	Urinating	-0.563	< 0.000*
Eating	Defecating	-0.557	< 0.000*
Fighting	Lying	0.051	0.407
Fighting	Standing	-0.140	0.023*
Fighting	Drinking	-0.167	0.007*
Fighting	Urinating	-0.072	0.243
Fighting	Defecating	-0.149	0.015*
Fighting	Eating	0.121	0.050*

*Statistically significant at p ≤ 0.05.

2005; Camerlink *et al.*, 2012).

Aggression was the first notable behaviour to be observed, as sows fought to establish their social hierarchy. Results presented in Figure 2 shows that aggression was significantly higher during the first day post weaning, then declined slowly with a stable environment obtained within three days. The behaviour performed by sows is in line with previous research, as it is well known that mixing unfamiliar weaning sows into group pens results in fighting as they established a relative social ranking (Arey, 1999; Barnett *et al.*, 2001)

Another notable increase in aggression was observed at post estrus, just after standing heat (9th and 10th days post estrus), as shown in Figure 2. A probable explanation may be that as a result of high sexual motivation, dominant sows might have permitted to be mounted by subordinate sows. However, an attempt of being mounted again outside estrus may result in aggression as dominant sows reclaimed their social ranking (Pedersen, 2007).

Excessive aggression can negatively impact on return-to-estrus by sows, as well as liter size (Mendl *et al.*, 1992; Barnett *et al.*, 2001; Marchant-Forde and Marchant-Forde, 2005). Fortunately, aggression was not found to be a major problem in the present study. This may be due to the fact that the sows were housed in small homogenous groups (5 sows per pen), were mostly multiparous, and probably had a previous experience of being in the same group, with a well-defined social hierarchy, as suggested by Hoy and Borberg (2009).

In an attempt to single out general behaviours that might be used to best describe the onset of estrus, a further analysis on Pearson linear correlation was run to check the existence of relationships among the variables, and the findings are presented in Table 2. The findings revealed that all the correlations were found to be statistically significant, except for fighting-lying and fighting-urinating.

A significant, negative strong correlation was found to exist between lying and standing (r = - 0.885, p ≤ 0.05), as shown in Table 2. As the onset of standing heat approached, the time spent on lying was decreased, while the standing time increased. Probably the increase on time spent on standing was explained by the various mating-related behaviours performed by weaning sows towards the onset of estrus. Another noteworthy observation was the significant positive strong correlation that existed between urinating and drinking (r = 0. 728, p ≤ 0.05). Recognizing that the study was conducted during the high ambient temperatures of the summer season, pigs might have resorted to frequent drinking as a self-cooling mechanism thus result in increased incidence of urination. Similar results were reported by De Oliveira Júnior *et al.* (2011), who explained that increased
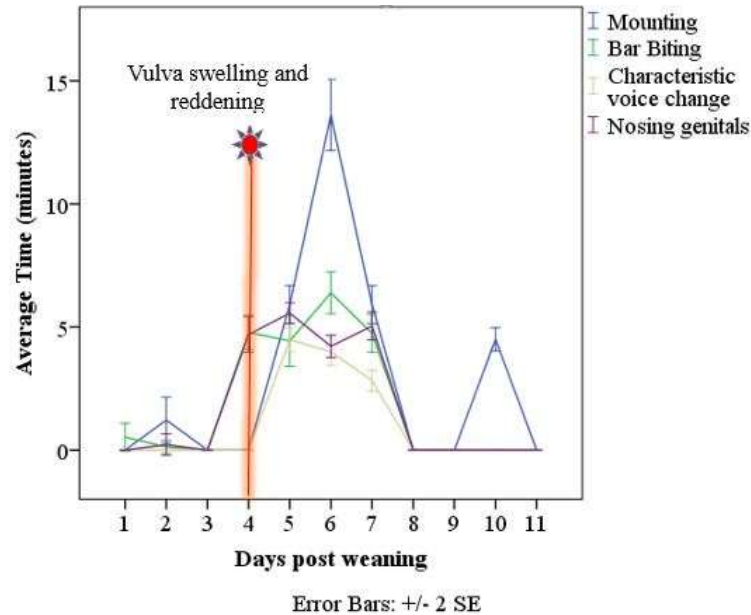
Figure 3. Variation in time duration of observed mating behaviours performed by weaning sows according to the days of the experiment, from weaning to post estrus (n = 32). **Note** that the total time of observation was 240 minutes per day.

consumption of water at high ambient temperatures was to compensate the thermal discomfort experienced by the pigs. Finally, a significant positive strong correlation (r = 0.719) was also reported between defecating and urination (p ≤ 0.05). It is believed that this association might be stimulated by the desire to mate, resulting from increased levels of oestrogen in the blood as sows approached standing heat.

Physiological changes of the vulva and sow replacement behavioural patterns associated with skill to mating observed before and after standing heat

Perhaps the most easily recognized and widely used symptom of estrus is vulva reddening and swelling (Seo et al., 2012; Johnson and Shade, 2017), however, it does not give conclusive results as it lacks the scientific evidence. Results in Figure 3 revealed that vulva swelling and reddening occurred approximately on the 4th day post weaning, and reached the peak on 5th day post weaning. Apparently, the findings revealed that the peak of vulva swelling and reddening coincided with increased physical activity among weaning sows. Geers et al. (1995) likened the magnitude of observed increase in physical activity to be almost ten times higher than that observed outside the estrus phase.

Although blood collection and hormone assay were not conducted in the present study, a potential of the

hormone changes can be drawn from existing literature. Different authors reported that vulva swelling and reddening were result of increased blood flow due to increased plasma estrogen (Abrams and Stolwijk, 1972; Abrams et al., 1973; Sterning, 1995; Langendijk et al., 2000; Simões et al., 2014).

It is believed that vulva secretions and sudden change in sow behaviour are associated with the varying concentration of gonadotropins found in pigs before and during estrus (Czaja and Butera, 1986; Knox, 2005; Knox, 2015). To further explain the hormonal influence of behaviour and vulva changes, Figure 4 provides an illustration of the relationships between the reproductive hormones, estrus and ovulations in pigs. When piglets stop suckling at weaning, the secretion of lactation hormones decreases and this marks increased levels of oestrogen from the growing follicles in the blood system, which is responsible for the expression of the sexual behaviour in sows (Ford, 1982; Ford, 1985; Pedersen, 2007; Knox, 2015).

In agreement with the aforementioned literature, the results presented in Figure 3 revealed that on the 4th day post weaning, a gradual increase in mating-related behaviours including mounting, bar biting, nosing genitals and characteristic voice change were observed. A summary of average time duration spent by sows performing the aforementioned mating-related behaviours is presented in Table 3.

It is important to note that, in all the observed mating-related behaviours, significantly higher average time
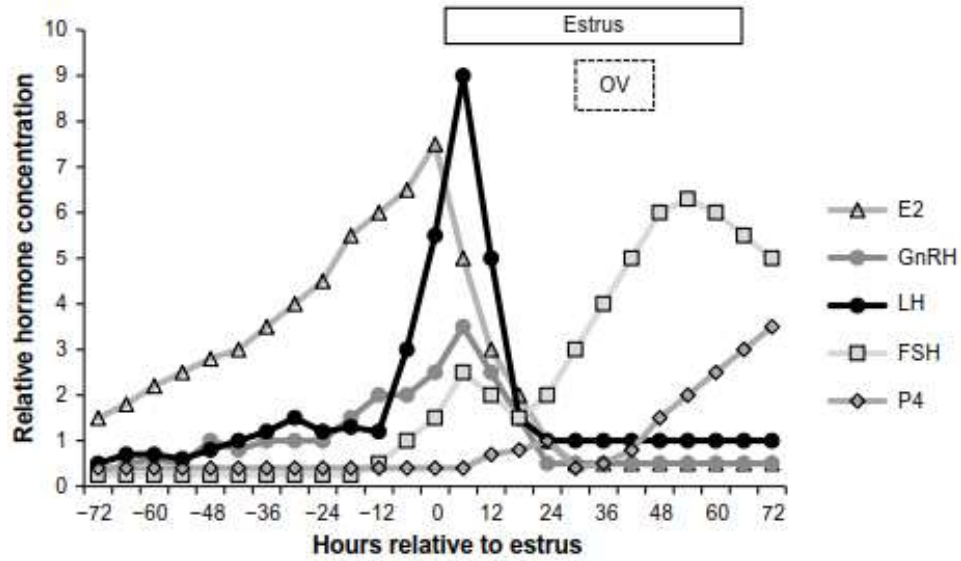
Figure 4. The diagrammatic illustration of hormonal profile during estrus and ovulation in pigs. **Note** Estradiol (E2), gonadotropin-releasing hormone (GnRH), Luteinizing hormone (LH), follicle-stimulating hormone (FSH), and progesterone (P4) during pro-estrus in relation to the timing of estrous and ovulation (OV) in the mature female pig. Source: Adapted from (Knox, 2015)

Table 3. Mean variation in time duration of all mating behaviours performed by sows according to the day of experiment, from weaning to post estrus, summer trial (n = 32).

	Mounting	Bar-biting	voice change	Nosing genitals
1st day	0.00[c]	0.00[c]	0.00[c]	0.00[c]
2nd day	1.22[c]	0.13[c]	0.00[c]	0.00[c]
3rd day	0.00[c]	0.00[c]	0.00[c]	0.00[c]
4th day	0.00[c]	4.78[b]	0.00[c]	4.70[b]
5th day	5.91[b]	4.74[b]	4.48[a]	5.57[a]
6th day	13.61[a]	6.39[a]	4.00[a]	4.22[b]
7th day	5.91[b]	4.78[b]	2.83[b]	5.04[ab]
8th day	0.00[c]	0.00[c]	0.00[c]	0.00[c]
9th day	0.00[c]	0.00[c]	0.00[c]	0.00[c]
10th day	4.50[b]	0.00[c]	0.00[c]	0.00[c]
11th day	0.00[c]	0.00[c]	0.00[c]	0.00[c]

[a, b, c]Means with different superscripts within columns are statistically different from each other (p ≤ 0.05).

duration (p ≤ 0.05) was found to be within the estrus phase (days 5 to 7 post weaning). Overall, weaning sows presumably mounted and allowed to be mounted, snout-sniffed genitals and body flanks, bar-bite, chanted and squealed as an attempt to attract the attention of the boar and stimulate its sexual activity, as suggested by different authors (Beach, 1976; Billings and Katz, 1999; Shearer and Katz, 2006). When sows were convinced that their

Table 4. Pairwise correlations coefficients (r) between time duration on all observed mating behaviours performed by sows during the time of the experiment (n = 32).

Variable 1	Variable 2	r	Probability
Bar biting	Mounting	0.610	< 0.000*
Characteristic voice change	Mounting	0.743	< 0.000*
Characteristic voice change	Bar biting	0.680	< 0.000*
Nosing genitals	Mounting	0.516	< 0.000*
Nosing genitals	Bar biting	0.809	< 0.000*
Nosing genitals	Characteristic voice change	0.731	< 0.000*

*Statistically significant at p ≤ 0.05.

attempts to attract the attention of the boar, the behaviours subdued in readiness for mating, accompanied with dropping levels of mating-related hormones in the blood, marking the beginning of true estrus (standing heat) at 6th day post weaning.

In line with several studies (Shearer and Katz, 2006; Johnson and Shade, 2017; Miura *et al.*, 2017), riding or mounting behaviour between pen mates has proved to be the most important signal of estrus in the present study. Mounting was the most observed replacement behaviour occurring for 13.61 minutes in every hour. Similarly, Pedersen (2007) reported that when mounting was observed over 3 days before commencement of estrus, it occurred 40 times per 24-hour period. Pedersen *et al.* (2003) further reported a significant relationship between riding behaviour and parity. These authors also shown that when comparing to primiparous sows, multiparous sows were found to be 8 times more likely to display mounting behaviour when on estrus.

Pairwise correlations between variables were run to single out proceptive behaviours that might be used to best predict the onset of estrus. Pairwise correlations between observed mating behaviours were all found to be significant and positive (p ≤ 0.05), as presented in Table 4. It is presumed that occurrence of nosing and bar biting among weaning sows is associated with increased likeliness of the onset of estrus. This assertion is based on the strong positive relationship that was found to exist between nosing genital and bar-biting (r = 0.809). The second most association that could be used by farmers to forecast the onset of estrus was the strong positive correlation between mounting and characteristic voice change (r = 0.743). Another recognised association was the positive strong correlation (r = 0.731) that existed between characteristic voice change and nosing genitals.

It is worth recognizing, that previous research reported mounting as the most reliable predictor variable for the onset of estrus (Shearer and Katz, 2006; Johnson and Shade, 2017; Miura *et al.*, 2017). However, pairwise correlations in the present study suggest that nosing genitals, bar-biting and characteristic voice change should not be ruled out as potential predictors of estrus. The results further suggest that perhaps weaning sows were mostly made of subordinate sows in the present

study. This study confirms the research work of Pedersen (2007). In their study, these authors reported a low female to female mounting when the population was mostly made of subordinate sows. Previously, Pedersen *et al.* (2003) had conducted a receptivity test on weaning sows and found that in response to courtships, more subordinate sows squealed than dominant sows.

Sow temperature variation at six anatomical body sites measured by days post weaning

In as far as using temperature variation as a predictive marker for estrus, previous studies reported contradictory findings, with some reporting increased body temperature, followed by a drop during estrus (Scolari *et al.*, 2011; Sykes *et al.*, 2012; Simões *et al.*, 2014).

Based on the findings of previous studies on sows and estrus detection, the present study hypothesized that as sows approached the onset of estrus, there will be an increase in sow activity and performance of various prospective behaviours accompanied by a rise and fall in sow body temperature. Indeed, after reddening and swelling of the vulva, as expected, the observed mating behaviours increased, along with a significant increase in body temperature.

Figure 5 shows temperature variation in the six anatomical body sites of weaning sows. The findings revealed a significant increase in all the measured body sites from the 4th day post weaning (-48 h), reaching the maximum temperature during the 5th day post weaning (-24 h). Temperature remained at peak for 48 hours during the summer trial, but only for 24 hours during the winter. The findings are in agreement with Simões *et al.* (2014), who reported an increased body temperature on sows during the pro-estrus phase. In their study, these authors reported a 3.8 ± 1.9°C increment in vulva-gluteal temperature 24 hours before the onset standing heat. Scolari *et al.* (2011) further suggested that the significance increase in vulva skin temperature prior to the onset of standing heat was due to vulva reddening and swelling emanating from high levels of estradiol hormone and increased blood flow.

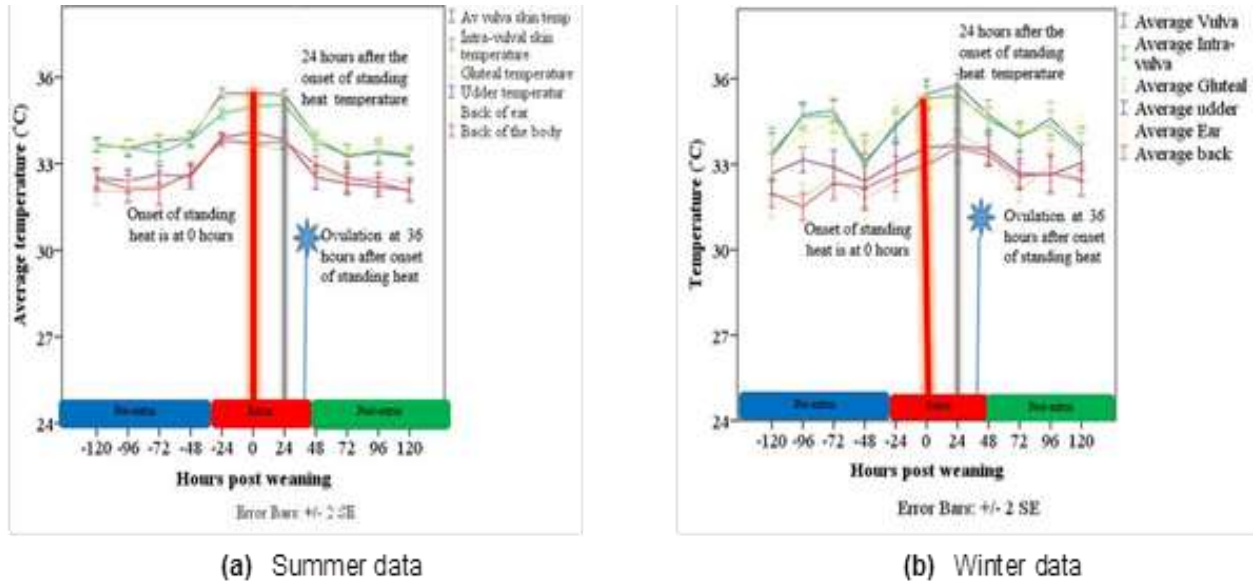Incidentally, peak temperatures measured at 5th and 6th

(a) Summer data   (b) Winter data

Figure 5. Mean temperature variation of weaning sows at six anatomical body sites measured by days post weaning.

Table 5. Pairwise correlations coefficients between temperature variation at six anatomical body regions measured on weaning sows during the time of the experiment.

Variable 1	Variable 2	Correlation		Probability	
		Summer	Winter	Summer	Winter
Intra-vulva skin	Vulva skin	0.675	0.817	< 0.000*	< 0.000*
Gluteal	Vulva skin	0.528	0.667	< 0.000*	< 0.000*
Back of ear	Vulva skin	0.579	0.403	< 0.000*	< 0.000*

*Means are statistically significant at p ≤ 0.05.

days post weaning (-24 and 0 h) shown in Figure 5, coincided with peak levels of estradiol previously shown in Figure 4. Furthermore, during these days that intensive display of proceptive behaviours such as mounting, nosing genitals and characteristic voice change were observed. Perhaps it was under the influence of estradiol that the aforementioned changes were observed. Several authors have previously concluded that temperature variation during estrus was probably a result of high levels of estradiol (Luño *et al.*, 2013; Simões *et al.*, 2014; Knox, 2015). Moreover, high levels of estradiol during estrus have been associated with the high incidence of sexual behaviours among sows (Ford, 1985; Knox, 2005; Knox, 2015). Consequently, it is during this phase (6th day post weaning or 0 hours) that sows responded positively to the back pressure test, marking the onset of true estrus which is considered the most important and reliable predictor of estrus in sows.

As the estrus phase progressed, vulva swelling and redness subdued, and this coincided with the decreasing levels of estradiol. Interestingly, the declining levels of estradiol hormone resulted in disappearance of sexual behaviour, and then notably, a significant drop in sow body temperature 24 hours after the onset of standing heat (Figure 5). The study shares the same sentiments with several studies that reported a reduced body temperature 12 to 24 hours prior to ovulation (Scolari *et al.*, 2011; Luño *et al.*, 2013; Simões *et al.*, 2014; Johnson and Shade, 2017). Luño *et al.* (2013) further suggested that the knowledge of the drop in body temperature 12 hours before ovulation provides a window to determine the time of ovulation in pigs. Perhaps farmers could use this drop in temperature as a right thumb rule that determines the best time to inseminate their sows, when conception rate is at maximum. Figure 5 further shows that during post estrus (96 hours) sow body temperature rose slightly, then stabilized to maintain normal body temperature, confirming the findings by Simões *et al.* (2014).

Table 5 shows selected pairwise correlations coefficients of mean temperatures obtained from the six anatomical body sites that could be used to best describe the onset of estrus in sows. Significant moderate and strong correlation for summer (r = 0.675) and winter (r = 0.817) trials, respectively, were found between intra-vulva and vulva skin temperatures. Consequently, as far as

temperature variation is concerned, these two variables were found to have a great potential to accurately predict the onset of estrus in weaning sows, as shown in Figure 5. This assertion was based on the scatter plot shown in Figure 5, which revealed a better fit to the data with $R^2 = 0.455$ and $R^2 = 0.667$ for the summer and winter trials, respectively.

Another recognised association shown in Table 5, was the moderate positive correlation that existed between gluteal and vulva skin temperatures in summer ($r = 0.528$) and during the winter season ($r = 0.667$). Findings from the present study further corroborates the findings from Luño et al. (2013). In their study, these authors reported a moderate positive correlation ($r = 0.60$) between vulva and ear skin temperatures, which however, was not significant. Coincidentally, the present study reported a significant moderate positive association between these two variables, $r = 0.579$ for summer, however, a positive weak association $r = 0.403$ during the winter season ($p \leq 0.05$).

CONCLUSION

The inverse relationship between time spent by weaning sows on standing and lying can be used as an indicator to alert farmers of approaching estrus, and a need for close inspection. Vulva swelling and reddening, increased physical activity and the increased likelihood of performance of mating behaviours by weaning sows can be used as markers to predict the onset of estrus. Besides the re-known symptoms of estrus in mounting, vulva swelling and reddening, the findings of the present study suggested nosing genitals, characteristic voice change and bar-biting as emerging potential predictors of estrus. To improve accuracy in estrus detection, the present protocol used behavioural and physiological changes of the vulva as primary diagnosis of estrus, and sows suspected to be on estrus were subjected to the boar test for further screening. Finally, to confirm the sows were indeed on standing heat, the back pressure test was conducted.

At onset of estrus, the performance of proceptive behaviours by weaning sows increased, accompanied by an increase in body temperature. True estrus (standing heat) was marked by the standing response either to boar or back pressure tests, suggesting that farmers with a small breeding herd have an opportunity to evade the cost of keeping a boar. This sequence of events and temperature variation at specified time frame provides a window to attach literature for accurate prediction of ovulation. Based on the finding of the study, it can be concluded that the flexibility of the infrared thermometer to be incorporated with other estrus detection protocols, yielded more accurate results, and could provide the small scale Hybrid Black pig production farmers with an opportunity to inseminate when the conception rates are at maximum.

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