

Observation on Estrous Synchronization Following Treatment with Prostaglandin in Crossbred Gilts

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Abstract: There exist a paucity of information on the usefulness of prostaglandins in the control of the pig's estrous cycle, perhaps, due to the brevity of the sensitive phase of its *corpora lutea* (CL). This study investigated the effects of three shot injections of 5mg, 7.5mg and 10mg prostaglandin F₂ alpha (Lutalyse®) at 7-day interval on induction of standing heat, as well as changes in vulva biometry and electrical conductance of vaginal mucus, in eighteen cycling crossbred gilts in three equal but separate groups (i.e. A, B and C). Two gilts in each group (i.e. control) received 2 ml phosphate buffered saline, while group-mates in A, B and C received 5mg, 7.5mg and 10mg Lutalyse®, respectively. Following the first; second; and third shots of Lutalyse®, 0%, 100% and 0% in group A, 50%, 50% and 0% in group B and, 0%, 50% and 0% in group C of gilts exhibited standing estrus. Similarly, 83.3% and 66.7% and 16.7% and 33.3% of treated and of control gilts showed and did not show estrus, respectively. The mean vulva area of gilts decreased ($P < 0.05$) from $13.71 \pm 2.96 \text{ cm}^2$ to $10.16 \pm 1.46 \text{ cm}^2$ during proestrus to diestrus, respectively. The electrical conductance of vaginal mucus changed ($P < 0.05$) from $331.25 \pm 66.03 \text{ U}$ to $418.13 \pm 34.53 \text{ U}$ and $397.50 \pm 80.07 \text{ U}$ during proestrus, estrus and diestrus, respectively. These results indicated that prostaglandins can synchronize estrus in pigs and two shots of 5mg Lutalyse® achieved the best (100%) synchronization. Similarly, evaluation of electrical conductance of vaginal mucus was more useful in staging estrous in gilts than vulva biometry.

Key words: Estrus Induction/Synchronization • Prostaglandin • Cycling Gilts

INTRODUCTION

Perhaps, the discovery of various protocols of estrus induction and synchronization of ovulation is one solution that science has proffered to circumvent the rigors and failures associated with estrus detection in animals. Several protocols involving the use of single agents (e.g. Prostaglandin F₂alpha) or combinations (e.g. Gonadotropin + Prostaglandin F₂alpha and, Progestins + Prostaglandin F₂alpha) are available in literature and have been reported to produce excellent results in the cow [1,2] sheep [3,4] and goat-doe[5-7]. In the case of sow/gilt, even though several literatures have claimed that single agents e.g. Regumate [8] and P.G. 600 [9] as well as combinations of certain agents are effective in synchronizing estrus. It was reported that there was still a paucity of approved protocols for swine [10]. Some of the methods that had also been claimed to

be effective for the swine include: use of norgestomet implant or a combination of estradiol benzoate and Prostaglandin F₂ alpha [11] combinations of gonadotropin and Prostaglandin F₂ alpha [12] as well as combinations of Regumate® and gonadotropin [13]. In spite of the advantages of estrus synchronization, the cost of the procedure is likely to be a central limitation to its adoption by farmers, especially small scale farmers in developing countries of sub-Saharan Africa. In a simple economic sense, protocols involving agent combinations might incur greater cost to the farmer as well as the consumer [14]. Apart from the issue of cost, it might be easier to search for a single product which is all sufficient by itself than looking for two or more products that either cannot be used, provided both protocols have comparable advantages. Other farm animals have benefited tremendously from the use of single agent such as Prostaglandin F₂alpha, for synchronization of estrus [15].

The swine, in spite of its high average litter size per sow [16] and more efficient carcass yield compared with cattle [17] is not known to benefit from Prostaglandin synchronization. The luteolytic activity of Prostaglandin F₂ alpha depends on the phase of maturity/functionality of the corpus luteum. During its three phases: refractory, sensitive and regressing, Prostaglandin F₂ alpha can only cause luteolysis during the sensitive phase of the CL's cycle [15]. This has been the fundamental issue with achieving synchronization with Prostaglandin F₂ alpha in all species. While other farm animals have a shorter refractory but longer sensitive periods, the opposite is the case with the swine [15]. The challenge therefore has been: how would Prostaglandin F₂ alpha be administered in order to target the sensitive phase of the corpus luteum in swine? Since the sow cycles every 21 days and its CL is refractory to Prostaglandin luteolysis within the first 12 or 13 days post ovulation [15]. This study was designed to investigate as well as compare the effects of administration of one-, two- (7 days apart) and three- (7 days apart) shots of 5 mg, 7.5 mg and 10 mg Prostaglandin F₂ alpha on standing estrus, vulva biometry and electrical resistivity of the vaginal mucus, in cycling gilts. The findings will contribute to knowledge in the area of assisted reproductive techniques with swine and possibly discover new and may, be cheaper or readily available method(s) of synchronizing estrus in swine, with the use of Prostaglandin F₂ alpha.

MATERIALS AND METHODS

Study Location: The study was carried out at the Piggery unit of the University of Ibadan Teaching and Research Farm (T&RF), Ibadan, Nigeria. Ibadan is the capital of Oyo State, South-western Nigeria and the city lies on Latitude 7°30' and Longitude 3°54'. The mean annual rainfall is 1250 mm and the city is classified under the sub-humid tropical climate. Minimum temperature is 21°C ± 1.5 while maximum temperature is 30°C ± 3.5 [18].

Animals and Management: Eighteen cycling crossbred (Landrace x Largewhite) gilts with age range 12–16 months and weighing 75 – 80kg were used in the study. The gilts were selected randomly, classified into three groups A, B and C and housed in different pens at another section of the T&R farm of the University. Each group had six gilts which were identified individually with the aid of Kruuse permanent marking stick around the head region. The gilts in each group were randomly

selected and numbered using Arabic numerals along with the corresponding group alphabet i.e. A1 – A6, B1 – B6 and C1 – C6. In each group, two gilts i.e. X5 and X6, where X represents the group identifier, served as control. The records obtained from the farm showed that the gilts were in good health and were cycling. The pens were made of non-slip concrete floors with walls and iron roofing sheets for shelter. The gilts were fed twice daily, in the mornings and evenings with a mixture of cassava peelings and concentrates. Clean water was made available *ad libitum* throughout the study.

Materials Used: The materials used were: Dinoprost Tromethamine (Lutalyse®), cotton wool soaked in methylated spirit, sterile needles, 5 ml and 10 ml syringes, Kruuse permanent marking stick, an inch and centimetre graduated ruler and the Draminski Estrous detector (Draminski, Owocowa, Poland).

Experimental Procedure: Three shots of PGF₂α (Lutalyse®) were administered intramuscularly (I.M.) to each of the gilts in the three groups. The gilts in groups A, B and C were given doses of 5mg, 7.5mg and 10mg Lutalyse®, respectively, with an exception of the control gilts in each of the group, which were treated with I.M. injections of 2 ml phosphate-buffered saline (PBS). The 3 shots were administered at 7days interval.

Determination of Estrus, Proestrus and Diestrus: The animals were observed twice during the day (8.00 a.m. and 4.00 p.m.) throughout the duration of the experiment for standing response when mounting is attempted by another gilt in the group. Gilts that stood still when being mounted were considered to be in estrus [19]. The duration of estrus was defined as a 48 hour period beginning from the moment standing response was observed. Proestrus was defined as a 72-hour period prior to the beginning of standing estrus. The duration of diestrus was defined as 72 hours after mounting attempts were refused.

These assumptions were based on Pond and Houpt's assertion that standing estrus i.e. the duration the pig will allow mating last for 2 – 3 days, diestrus last for 15 – 18 days and proestrus 1 – 3 days.

Evaluation of Standing Estrus: The standing estrus i.e. immobilization in response to back pressure from a boar, another sow or a person; which is the ultimate and most effective way of detecting estrus [20] was used to ascertain estrus.

Observation of the gilts for standing heat commenced a day after the first administration of Lutalyse®. The gilts were monitored twice daily, in the mornings and evenings for at least one hour each time to detect the gilts that exhibited the standing response and observations were recorded.

Gilts that stood to be mounted by other gilts were considered to be in estrus, while gilts that exhibited mounting attempts on other gilts were considered to be in proestrus. Mountings that occurred for at least one minute were taken as positive. The percentage of animals that stood to be mounted in their respective groups after each administration of Lutalyse® was recorded.

Evaluation of Vulva Biometry: The diameters of the vulva i.e. vertical (Distance between superior and inferior commissures) and horizontal (At the broadest horizontal curvature) were measured using a 30 cm long transparent ruler which was lightly placed against the vulva lips, to avoid pressure, of both control and treated gilts. The vulva area was thereafter calculated as shown below:

$$\text{Vulva area (cm}^2\text{)} = \text{Length of vertical diameter (cm)} \times \text{Length of horizontal diameter (cm)}.$$

Evaluation of Vaginal Electrical Conductance/Resistance: The Draminski Estrous detector was manufactured for the evaluation of the electrical resistance of vaginal mucus which has been observed to change with the different phases of the estrous cycle [21]. The equipment was used according to manufacturer’s instruction. Gel was applied to lubricate the probe prior to vaginal insertion. The probe was then rolled thrice and gently against the vagina mucosa, pressing the knob after each twist.

At the end of each exercise, the measurement device will visibly indicate the phases as actual potential calibrated to milli-volts from a circuit formed of solid state and low noise elements in which a high input impedance is combined with high common mode rejection with significant processing techniques which includes input filtering to exclude extraneous as well as body induced currents. This was done three times on individual experimental animals as well as controls and readings were recorded.

Data Collection: All procedures leading to data collection was carried out daily, starting from the second day following each injection with Lutalyse® under the three protocols i.e. 5 mg, 7.5 mg and 10 mg, respectively.

Ethical Approval: The study was carried out under strict adherence to the guidelines of the Animal Care, Use and Research Committee (ACUREC) of the University of Ibadan, Nigeria.

Statistical Analysis: Data collected was analyzed using the Statistical Package for Social Sciences (SPSS 19) and presented as percentages or proportions and Mean ± S.D. The student-t-test and Analysis of variance were also used. Results were considered significant at P < 0.05.

RESULTS AND DISCUSSIONS

Standing Estrus: The results in Tables 1 and 2 indicates that Lutalyse® has induced, as well as synchronized estrus in treated gilts. It is however important that before interpreting the implications of observations with respect to standing estrus in this study. It should be recalled that the basic rule guiding the mechanism of action of

Table 1: Proportion of standing estrus following three shots of Lutalyse® administration to gilts.

Lutalyse® injection (s):	Gilts in estrus (%) in Group A (5 mg)	Gilts in estrus (%) in Group B (7.5 mg)	Gilts in estrus (%) in Group C (10 mg)
First shot	0	50	0
Second shot	100	50	50
Third shot	0	0	0
Total	100	100	50

Table 2: Summary of proportions of gilts that either showed or failed to show standing estrus.

Gilt Group	Proportion that showed standing estrus	Proportion that failed to show standing Estrus	Significance
Treated (administered Lutalyse®)	83.3 %	16.7 %	P<0.05
Control (administered PBS)	66.7 %	33.3 %	P<0.05

prostaglandin will subsist i.e. treated animals needed to possess a functional or responsive *corpus luteum/corpora lutea* [1] to be effective. Although, none of the gilts in group A (Injected with 5 mg PGF_{2α}) came into estrus following the first shot of PGF_{2α} injection, all (i.e. 100 %) showed estrus following the second shot/dose. While the observation following the first shot may be due to the physiological status of the *corpora lutea* in that they may be in either the refractory or regressing phase [15]. Observation following the second shot suggests not only that 5 mg Lutalyse® is capable of synchronizing estrus in gilts, but also counteracts earlier claims that PGF_{2α} alone could not synchronize estrus in gilts [22,23]. Similarly, following the third shot of PGF_{2α} injection, none of the gilts in group A came into estrus. This is expected since 100 % of the gilts already came into estrus following the second PGF_{2α} injection. The CL at the time of the third shot of PGF_{2α} in this study, is likely to be in the refractory phase [15]. In group B (i.e. 7.5 mg PGF_{2α}), 50 % of the gilts came into estrus following the first shot of PGF_{2α} injection. This result suggests that the CL of 50 % of the gilts were in the responsive phase of their cycle while those for remaining 50 % were either in the refractory or regressing phase. Following the second shot of PGF_{2α} injection, the remaining 50% came into estrus, suggesting that those *corpora lutea* that were not responsive at the time of the first shot of Lutalyse® were then responsive. Again, as with the 5 mg group, none of the gilts came into estrus in group B following the third shot/dose of PGF_{2α}. In group C (i.e. 10mg PGF_{2α}), none of the gilts came into estrus after the first shot of PGF_{2α}. This observation suggests that none of the CLs of gilts under the 10 mg Lutalyse® was in the responsive phase as at the time of the first shot, probably due to earlier stated reasons. Following the second shot, 50 % of the gilts were observed in standing estrus, suggesting that the CLs of 50 % of the gilts were then in the responsive phase at the time of that injection. However, following the third shot/dose of the PGF_{2α}, none of the gilts was seen showing standing estrus. The finding with gilts treated with 10 mg Lutalyse® was completely different from the earlier two groups in that only 50% of the gilts showed estrus. In comparison with other *species* in which Lutalyse® had been used to synchronize estrus, this proportion (i.e. 50 %) is less than 83.3% and 90 % reported in goat does [6, 24] as well as 60-70% reported for cattle [25] suggesting probably, that prostaglandins are less effective in gilts at 10 mg dosage when compared with earlier results at 5 mg and 7.5 mg dosages. Knowing that Lutalyse® is luteolytic [26] could the CL of these 50 %

gilts in Group C have escaped PGF_{2α} action?, the scope of the current study may fail to provide an answer to this query. However, it was reported that PGF_{2α} is rapidly metabolized by the lungs, liver and kidney [27]. It is thought that if the rate of metabolism of PGF_{2α} increases in this group of gilts, then PGF_{2α} may fail to exercise its luteolytic effect on such gilts. Again, considering the limits of this study, the three shots of Lutalyse® were administered within 14 days in which following the third shot/dose, observation began 24 hours after and lasted for 96 hours, implying that the entire study lasted for 18 days. This study period left open a window of about 72 hours of the estrous cycle length of the gilt within which other vital observations may be noticed. Looking across the rows in Table 1, following the first shot of PGF_{2α}, 0 %, 50 % and 0 % came into estrus in groups A, B and C, respectively. Following the second shot, 100 %, 50 % and 50 % of the gilts came into estrus in groups A, B and C, respectively. Similarly, following the third shot, none of the gilts came into estrus in all treatment groups. These observations suggest that the third shot is not effective in synchronizing estrus in the gilts. Two shots however appears to be the best protocol since the greatest percentage of the gilts came into estrus after that treatment. The first shot caused some level of synchronization, with just a very little proportion of the gilts in estrus following the single injection as observed with the 7.5 mg group. Table 2 further shows that 66.7 % of control gilts showed standing estrus during the period of the study. This observation validates information collected from the records that the gilts were cycling. However, it is not impossible that the 33.3 % which failed to show standing estrus would do so during the period not captured in this study.

Vulva Biometry: In Table 3, serial No. 1 compared changes in the vertical diameters of the vulva of gilts within the same treatment group (i.e. 5 mg). The result suggest that the pattern and extent of changes were similar within the 5 mg treatment group. This observation is also true with the 10 mg treatment group as shown by serial No. 10. The situation is however different with the 7.5 mg group as shown by serial No. 6. Although, the current study may not provide an explanation for this disparity, it suggest that a more intense response to Lutalyse® by the some of the gilts in the 7.5 mg group may be responsible. Results on serial Nos. 2, 3, 5, 7, 8, 11 and 13 compared within same treatment groups, values of and changes between vertical and horizontal diameters and shows that the vertical diameters were larger (P<0.05)

Table 3: Comparison of means of vulva biometrical changes within treatment groups.

Vulva Diameter Pairs	Significance
1.vdva1 - vdva3	P>0.05
2.vdva1 - vdha1	P<0.05
3.vdva3 - vdha3	P<0.05
4.vdha1 - vdha3	P<0.05
5.vdvac - vdha3	P<0.05
6.vdvb1 - vdvb3	P<0.05
7.vdvb1 - vdhb1	P<0.05
8.vdvb3 - vdhb3	P<0.05
9.vdha1 - vdhb3	P<0.05
10. vdvc4 - vdvc3	P>0.05
11.vdvc4 - vdhc4	P<0.05
12.vdhc4 - vdhc3	P<0.05
13.vdhc3 - vdvc3	P<0.05

Table 4: Comparison of vulva areas (cm²) between different gilt groups and stages of the cycle.

Mean ± S.D. (Abbreviated stage of estrous)	Mean ± S.D. (Abbreviated stage of estrous)	P-value
13.71±2.96 (Proheat)	12.18±3.25 (Proaac)	P>0.05
13.71±2.96 (Proheat)	10.98±1.18 (Prono)	P>0.05
13.71±2.96 (Proheat)	11.85±2.08 (Estruheat)	P>0.05
13.71±2.96 (Proheat)	10.16±1.46 (Diestrheat)	P<0.05
11.85±2.08 (Estruheat)	10.16±1.46 (Diestrheat)	P>0.05
11.85±2.08 (Estruheat)	13.50±0.71 (Estruaac)	P>0.05
11.85±2.08 (Estruheat)	11.04±0.34 (Estruno)	P>0.05
10.16±1.46 (Diestrheat)	12.83±1.06 (Diestrno)	P>0.05
10.16±1.46 (Diestrheat)	14.60±0.53 (Diestraac)	P<0.05

than their horizontal counterparts. This is similar to a recent report in *Bunaji* cows [28]. Results on serial Nos. 4, 9 and 12 similarly compared changes in horizontal diameters within same treatment groups and shows that the changes that occurred were not at comparable rates. This finding will suggest that individual gilts exhibited varying degrees of response to Lutalyse® with respect to changes in their vulva diameters and therefore limit the reliability on the usefulness of the parameter in predicting estrus. This would suggest that changes in vulva diameters of the gilts, with respect to staging estrous, are comparable with some other *species* e.g. goat [6] and cattle [29] in which it had been reported as imprecise. However, Table 4 compared vulva areas (cm²) between different gilt groups and stages of the cycle. The table shows that the difference between mean vulva areas, of gilts which showed standing estrus, during proestrus i.e. Proheat (13.71±2.96) cm² and diestrus i.e. Diestrheat (10.16±1.46) cm² was significant (P<0.05). Since the

differences between proestrus and estrus i.e. Estruheat, as well as estrus and diestrus were not significant (P>0.05), the clinical relevance of relying on vulva biometry in staging estrous appears to be narrowed to distinguishing only between proestrus and diestrus, from the current study. This finding at least suggest that credence may be given to vulva biometry in staging estrous in pigs compared to some other *species* earlier mentioned. The mean diestrus vulva area (10.16±1.46 cm²) was also found to be lower (P<0.05) than its corresponding value in control gilts (14.60±0.53 cm²). Perhaps the only clinical relevance of this finding is that it tells a difference between treated and untreated gilts.

Vaginal Electrical Resistance (Units): The clinical usefulness of the Draminski estrous detector in staging estrous in gilts appears to be quite broad, going by the results of the current study in Table 5. This is because it shows that the differences between all the mean ± S.D. pairs compared were significant (P<0.05). For example, between the proestrus i.e. Proesheat of treated gilts in heat (331.25±66.03 U) and control gilts i.e. Proeactr (283.33±40.10 U) or treated gilts that failed to show standing estrus i.e. Proenoheat (371.67±20.21 U) or standing estrus in treated gilts i.e. Esthtod (418.13±34.53 U) and diestrus in treated gilts i.e. Dieheatod (397.50±80.07 U), the differences were significant (P<0.05). Similarly, between standing estrus in treated gilts and diestrus (397.50±80.07 U) or the period corresponding to estrus both in control (272.50±80.07 U) and treated gilts that failed to show estrus (322.50±74.25 U), the differences were significant (P<0.05). Also, between the diestrus value in treated gilts that showed standing estrus and the period corresponding to diestrus in both control gilts (341.67±59.65 U) as well as treated gilts which failed to show estrus (430.00±57.66 U), the differences were significant (P<0.05). These results clearly suggest that the Draminski estrous detector is capable of unequivocally staging estrous in the pig. It is clear from Table 5 that the mucus resistance rose from a proestrus value of 331.25±66.03 U to 418.13±34.53 U, during estrus and declined to 397.50±80.07 U, during diestrus. Although, this pattern is not same as found in heifer and sheep, where the estrus values were reported as lowest (P<0.05), compared with other stages, since the differences in the mean values of the current study were significant (P<0.05), the Draminski estrous detector appears to be useful in staging estrous in the pig. It is also probable that the non-similar pattern may be due to *species* differences and also, differences with respect to electrical

Table 5: Comparison of vaginal electrical conductance between gilt groups and stages of the cycle.

Mean ± S.D. (U) (Abbreviated stage of estrous)	Mean ± S.D. (U) (Abbreviated stage of estrous)	P-value
331.25±66.03 (Proesheat)	283.33±40.10 (Proeactr)	P<0.05
331.25±66.03 (Proesheat)	371.67±20.21 (Proenoheat)	P<0.05
331.25±66.03 (Proesheat)	418.13±34.53 (Esthtod)	P<0.05
331.25±66.03 (Proesheat)	397.50±80.07 (Dieheatod)	P<0.05
418.13±34.53 (Esthtod)	397.50±80.07 (Dieheatod)	P<0.05
418.13±34.53 (Esthtod)	272.50±80.07 (Estacod)	P<0.05
418.13±34.53 (Esthtod)	322.50±74.25 (Estnohtod)	P<0.05
397.50±80.07 (Dieheatod)	430.00±57.66 (Dienheatod)	P<0.05
397.50±80.07 (Dieheatod)	341.67±59.65 (Dieactrod)	P<0.05

conductance during different stages of the estrous cycle in the pig. Since previous studies [21, 30, 31] have laid claims to the clinical relevance of the Draminski estrous detector in staging estrous in various *species* of animals, the observation in the current study becomes informative as credence is been laid to the ability of the equipment in staging estrous in cycling gilts and more so, it confirms the proposition that electrical conductance of vaginal mucosa may be useful in determining optimum insemination time in pigs [32].

CONCLUSION

The administration of two doses of PGF_{2α} (5mg/gilt) intramuscularly, 7 days apart achieved estrus in 100% of gilts. Hence the 2-shot protocol using 5mg/giltPGF_{2α} is the recommended protocol for estrus synchronization in gilts using Lutalyse® (i.e. PGF_{2α}). Similarly, vulva biometry is not as effective as electrical conductance of vaginal mucus in staging estrous in gilts.

Further studies would be designed to span the entire length of the pig's estrous cycle with greater number of gilts.

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