ISSN 0189-0514
J. Anim. Prod. Res. (2017) 29(1):63-69

National Animal Production Research Institute
Ahmadu Bello University
P.M.B 1096, Shika-Zaria,
Kaduna State, Nigeria.
Email:japr@napri.gov.ng
Website: http://journals.napri.gov.ng

PROGESTERONE PROFILES OF CROSSBRED SOWS FOLLOWING DOUBLE AND TRIPLE INJECTIONS OF CLOPROSTENOL SODIUM (SYNCHROMATE®)

***[1]Oke-Egbodo, B. E., [3]Bawa, E. K., [4]Nwannenna, A. I., [4]Oke, P. O., and [3]Hassan, R.**

[1]Swine and Rabbit Research Programme, National Animal Production Research Institute, Ahmadu Bello University, Zaria-Nigeria [2]Biotechnology Research Programme, [2]Department of Theriogenology and Production, Faculty of Veterinary Medicine, Ahmadu Bello University, Zaria-Nigeria.

[3]Biotechnology Research Programme, National Animal Production Research Institute, Ahmadu Bello University, Zaria-Nigeria

[4]Department of Parasitology and Entomology, College of Veterinary Medicine, Federal University of Agriculture, Makurdi-Nigeria.

**Corresponding mail: engodvm@yahoo.com Phone: +2347033227982),*

ABSTRACT

This study was carried out to evaluate progesterone profile of crossbred sows following treatments with double and triple injections of Cloprostenol sodium (Synchromate®). Ten (10) apparently healthy crossbred sows were divided into 2 groups. The groups were randomly assigned to 2 treatments based on number of injections of Synchromate®. Group 1 (n=5) received two injections of Synchromate® (500µg) (on days 0 and 13) while Group 2 (n=5) received three injections of Synchromate® (on day 0, 7, 13). Oestrus was monitored twice daily from 0700 – 1000 and 1500 – 1800h. Data on progesterone profile using competitive ELISA kits. The sensitivity of the assay was 0.105 ng/ml. Within assay precision, coefficients of variation for low, normal and high pooled controlled serum samples were 9.9%, 3.1% and 2.9% respectively were expressed as mean ± SEM. Student t-test was used to compare the mean values between the groups. Graphpad Prism® data package for windows (2009) was employed for all statistical analyses. A value of $P < 0.05$ was considered significant. There was no statistical significant ($P > 0.05$) difference in the overall mean serum P4 concentrations between groups 1 and 2. It is concluded that cloprostenol sodium can be used to synchronize sow oestrus, both double and triple injections of cloprostenol sodium produces similar effect on progesterone concentration. It is recommended that double injections of cloprostenol sodium should be used for oestrus synchronization as no statistical significant ($P>0.05$) differences were observed in the progesterone concentrations of both groups.

Keywords: Progesterone concentration, Cloprostenol sodium, crossbred sows, Oestrus, Synchronization

INTRODUCTION

Pig production represents the fastest way of increasing animal protein since pigs grow at a faster rate and reproduce sooner with large number of offspring than cattle, sheep or goats (Ajala, 2003; McGlone, 2013). They have short generation interval, high production potential, high prolificacy, high carcass yield and can adapt easily to environmental conditions (ILCA, 1992; Osaro, 1995; Vicente *et al*., 2011). Therefore, pig production has been advocated as a short term measure towards alleviating the animal protein and calorie deficit, especially where there are no religious edicts preventing their production and consumption, (Ajala *et al.*, 2007).

Understanding the regulation of the oestrous cycle is essential for successful implementation of a synchronization protocol which allows manipulation of oestrus and ovulation hence allowing a fixed-time artificial insemination or natural mating which is of great benefit for pig farms that operate using batch farrowing (Brown, 2006).

Prostaglandin $F_{2\alpha}$ ($PGF_{2\alpha}$) is not luteolytic in the sows until about day 12 of the oestrous cycle (De Rensis *et al*., 2012; Kouamo and Kamga-Waladjo, 2013; Tur, 2013). The insensitivity of sow's CL to the luteolytic effect of $PGF_{2\alpha}$ before day 12 is considered to preclude the use of prostaglandin in oestrus synchronization programmes for swine (Przygrodzka *et al*., 2015).

In swine, ELISAs have been used for determining pregnancy via progesterone (P4) concentrations in serum, plasma, blood, saliva, and feces (Sanders *et al*., 1994; Moriyoshi *et al*., 1997). Serum P4 concentrations measure luteal function and, thus, the pregnancy and oestrous status of the sow (Althouse and Hixon, 1997). To the best of our knowledge, little or no study has evaluated the progesterone profile of crossbred sows treated with double (days 0 and 13) and triple injections (days 0, 7 and 13) of Cloprostenol sodium (Synchromate®). The underlying objective of this study was to check for progesterone profile using competitive ELISA following double injections (days 0 and 13) and triple injections (days 0, 7 and 13) of Cloprostenol sodium (Synchromate®) injections.

OBJECTIVE/AIM

To evaluate the progesterone profile/ concentration using competitive ELISA following double and triple injections of Cloprostenol sodium and its luteolytic effects on days 7 and 13 of the oestrous cycle.

MATERIALS AND METHODS

Study Location

The study was carried out at the Swine Farm Unit of the Swine and Rabbit Research Programme, National Animal Production Research Institute (NAPRI), Shika, Ahmadu Bello University, Zaria. Zaria is located in the Northern Guinea Savannah zone of Nigeria between latitude 11°N and 12°N and between longitude 7°E and 8°E at an elevation of 650 m above sea level. The average annual maximum and minimum temperature are 31.0 ± 3.2°C and 18.0 ± 3.7°C respectively and an average annual rainfall of 1100 mm usually lasting from May to October with a mean relative humidity of 72% while the dry season lasts from November to April with daily temperatures of 15 - 36°C and mean relative humidity of between 20 – 37% (Kowell, 1986; Sawa and Buhari, 2011).

Experimental Animals and Herd Management

Ten (n = 10) apparently healthy crossbred sows belonging to the Swine and Rabbit Research Programme of the NAPRI Shika, Zaria were used for this study. The sows used in this study were between the age of 2 – 3, years weighed between 120 and 150 kg. The sows were identified by ear tag numbers.

The crossbred sows were fed with diet containing 16% crude protein. The ration was formulated to meet the minimum nutrient requirements for breeding sows and boars as recommended by National Research Council (NRC) (1998). The ingredients for the diet were sourced in NAPRI

feed store and the ration was mixed in the feed mill in NAPRI, Shika, Zaria. Water was given *ad libitum.*

Experimental Design
A total of ten (n = 10) crossbred sows were randomly divided into two groups

The experimental group.
A total of ten (n = 10) crossbred sows were divided into two groups and the groups randomly assigned to one of two treatment protocols as described below:

Group 1 (n=5) – Double intramuscular injection of Cloprostenol sodium (Synchromate®).

Each of the sows received a dose of 500 µg (2 ml) cloprostenol sodium injection on days 0 (day of first injection) and 13 (day of second injection). The sows were then monitored for signs of oestrus. Those found in oestrus were bred using natural breeding.

Group 2 (n=5) – Triple intramuscular injection of Cloprostenol sodium (Synchromate®).

Each of the sows received a dose of 500 µg (2 ml) of cloprostenol sodium on days 0 (day of first injection), 7(day of second injection) and 13 (day of third injection). The sows were also monitored for signs of oestrus (Figure 3.1). Those found exhibiting signs of oestrus were bred using natural mating.

Oestrus Detection and Mating
The crossbred sows were observed visually for behavioural oestrus manifestation twice (0700-1000 and 1500-1800 h) daily from commencement of the study for 21 days. Sows were considered to be in oestrus when they stood to be mounted by females (homosexual mount) or male (heterosexual mount).

Blood Sampling
Five (5) milliliters of blood was collected via the posterior vena cava, using a 10-ml hypodermic syringe, fitted with 18 gauge needle, from the sows on days 0, 7, and 13 (just before prostaglandin injection), and once weekly afterwards until confirmation of pregnancy based on non-return to oestrus. Blood samples collected in vacutainers without anticoagulant were quickly transported to the laboratory. Serum samples were separated by centrifugation of the blood at 2500G for 15 minutes. The serum samples in vials were appropriately labeled and stored at -20°C until hormone analysis.

Progesterone Assay and Test Procedure
Serum P4 was determined by using Competitive ELISA kits (AccuBind® ELISA, Monobind Inc. 100 North Pointe Drive Lake Forest, CA 92630, USA), intended for a quantitative determination of P4 concentration in serum or plasma using ELISA microplate reader (ELx800). The sensitivity of the assay was 0.105 ng/ml. Within assay precision, coefficients of variation for low, normal and high pooled controlled serum samples were 9.9%, 3.1% and 2.9% respectively.

Before commencing the procedure, all reagents, serum references for P4 and samples were brought to room temperature (25 ± 2˚c). 25 µL of each serum reference for progesterone (0.0, 0.3, 2.0, 5.0, 15.0, 30.0 and 60.0 ng/ml) were and coated in duplicate wells A1 through B2 (A1-A12, B1-B2). 25 µL of respective serum samples were pipetted into wells B3 through H12 (B3-B12, C1 – C12, D1 – D12, E1-E12, F1-F12, G1-G12, H1-H12). 50 µL of progesterone enzyme reagent were then pipetted and added to all the wells. The content was mixed by gentle swirling for 20 seconds. Another 50 µL of progesterone biotin reagent were pipetted and added into all

wells. Content were swirled for another 20 seconds. The wells then covered with a foil and incubated at room temperature for 60 minutes. After the incubation, the

i. Content were discarded by decantation and the plate was blot dried with absorbent tissue paper. The plate was washed 3 times by tapping and blot drying on a tissue paper following addition of 350µL of wash buffer in each well before each wash.

ii. 100 µL of substrate solution were added to all wells without shaking the plate and Incubated at room temperature for 20 minutes. 50 µL of stop solution was finally added to each well and gently mixed for 15-20 seconds.

iii. Absorbance for each well was read at 450 nm wavelength ELx800 microplate reader within 20 minutes of adding stop solution. Absorbance from micro reader was subjected to a MasterPlex® ReaderFit ELISA analysis software from which absorbance for each duplicate serum reference versus corresponding progesterone concentration in ng/ml was plotted. A best-fit curve was automatically obtained. Progesterone concentration in ng/ml for each unknown sample was interpolated automatically from best-fit curve.

Data Analyses

Data on progesterone profile during the oestrous cycle were expressed as mean ± SEM. Student t-test was used to compare the mean values between the groups. Graphpad Prism® data package for Windows (2009) was employed for all statistical analyses. A value of $P < 0.05$ was considered significant.

RESULTS AND DISCUSSSION

In group 1, the basal concentration of P4 (3.77 ± 1.53 ng/ml) after the injection of $PGF_{2\alpha}$ was an increased (6.50 ± 0.20 ng/ml) after one week of the injection (figure 1) this shows that most of the animals in group 1 were in their early stage of luteal phase making the CL unresponsive to the injected $PGF_{2\alpha}$ as porcine CL is unresponsive to exogenous $PGF_{2\alpha}$ before day 12 which is in agreement with Guthrie and Polge, 1976, Estill *et al*., 1993, Zannoni *et al*., 2007, De Rensis *et al*., 2012 and Kouamo and Kamga, 2013. There was luteolys following second dose of the $PGF_{2\alpha}$ at day 13 (0.63 ± 0.09 ng/ml) with oestrus signs which is in corroboration with the luteolytic effect of $PGF_{2\alpha}$ on porcine CL as reported by De Rensis *et al*., 2012. Pregnancy was established following increased P4 concentration (11.87 ± 0.20 ng/ml) as shown in table 1, which is in agreement with Boma and Bilkei (2008) who reported P4 concentration of pregnant sows to be greater than 5ng/ml.

In group 2 with triple doses of synchromate®, the basal p4 level (7.13 ± 0.64 ng/ml) decreased to (4.43± 1.72 ng/ml) which indicate that the most of the animals were in their late luteal phase on the day of first $PGF_{2\alpha}$ injection which was able to cause decrease in P4 concentration following the first dose of the injection but the second dose given on day 7 (4.43± 1.72 ng/ml) (Figure 1) there was no reduction in the p4 rather an increased P4 was seen (6.57 ± 0.23 ng/ml) then a decrease in the p4 (0.23 ± 0.09 ng/ml) following the third dose of $PGF_{2\alpha}$ due to the CL responsiveness to $PGF_{2\alpha}$ from day 12 as reported by Estill *et al*., 1993 and Kouamo and Kamga, 2013 and oestrus was observed in the animals hence complete luteolysis occurred after the third injection of $PGF_{2\alpha}$ as shown in table 1. Following oestrus and natural breeding there was significant increase in the p4 concentration from mating till day 48 (11.93 ± 0.87 ng/ml) which indicates pregnancy as reported by (Boma and Bilkei, 2008).

Table 1: Progesterone profile of crossbred sows treated with double and triple injections of Cloprostenol sodium (Synchromate®).

Days	Grp 1 (n = 5)	Grp 2 (n = 5)
0	3.77 ± 1.53	7.13 ± 0.64
7	6.50 ± 0.20	4.43± 1.72
13	6.73 ± 0.29	6.57 ± 0.23
20	0.63 ± 0.09	0.23 ± 0.09
27	6.60 ± 1.41	6.10 ± 1.01
34	8.73 ± 1.34	8.97 ± 1.52
41	9.40 ± 0.70	10 ± 1.00
48	11.87 ± 0.20	11.93 ± 0.87

n=number of animals in each group. There was no statistical significant (P > 0.05) difference in the overall mean serum P4 concentrations.

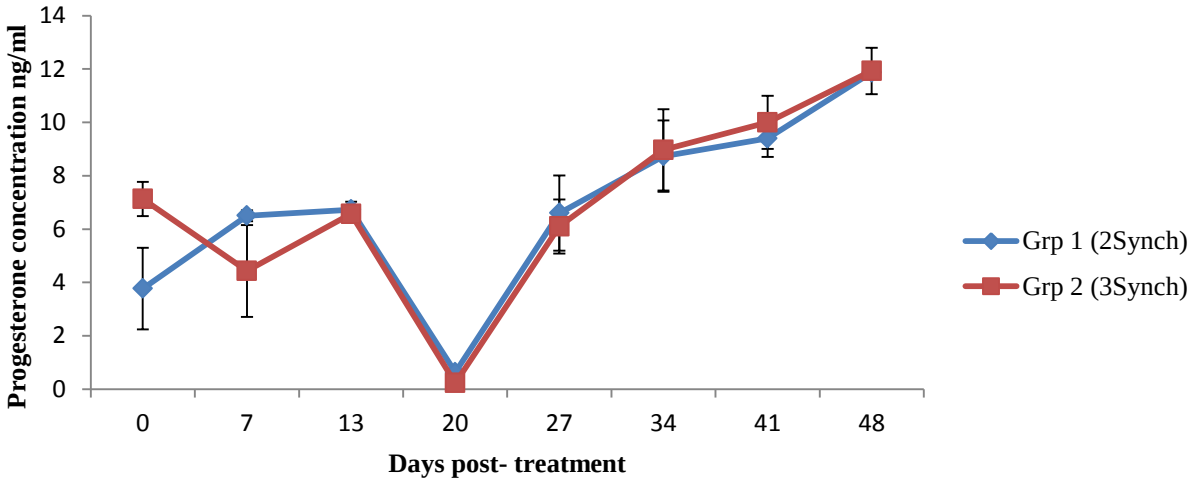


Figure 1: Progesterone profile of sows treated Synchromate® (Cloprostenol sodium).

KEYS

Grp 1 = crossbred sows treated with double injection of Cloprostenol sodium at days 0 and 13

Grp 2 = crossbred sows treated with triple injection of Cloprostenol sodium at days 0, 7 and 13

CONCLUSION AND RECOMMENDATION

From the results of this study, it can be concluded that:

1. Cloprostenol sodium (Synchromate®) has luteolytic effect on porcine CL and can be used to synchronize sows to enhance productivity.

2. There was significant difference in the effect of double injection and triple injection of exogenous $PGF_{2\alpha}$ (Synchromate®) on progesterone concentrations in swine. It is therefore recommended that Double injection of Cloprostenol sodium (Synchromate®) at 13 days protocol should be by farmers to save cost and labour.

ACKNOWLEDGEMENT

The authors would like to express their profound gratitude to the Director, National Animal Production Research Institute, Olorundare Emmanuel, Ohaba Yusuf and Ede Peter of Swine and Rabbit Research Programme and All Staff of Hormonal Laboratory of Biotechnology Research Programme NAPRI, Ahmadu Bello University, Zaria.

REFERENCES

Ajala M. K. (2003). Economics of swine production in Jama'a Local Government Area of Kaduna State, Nigeria. *Tropical Journal of Animal Science*, 6 (1): 53-62.

Ajala, M. K., Adesehinwa, A. O. K. and Mohammed, A. K. (2007). Characteristics of smallholder pig production in Southern Kaduna area of Kaduna State, Nigeria, *American-Eurasian Journal of Agriculture and Environmental Science*, 2: 182-188.

Althouse, G. C. and Hixon, J. E. (1999). Use of commercially available ELISAs to help determine oestrous status in female swine. *Swine Health and Production*, 7(2): 65-68.

Boma, M. H. and Bilkei, G. (2008). Field experience with early pregnancy diagnosis by progesterone based ELISA in sows. *Ondersterpoort Journal of Veterinary Research*, 75: 55-58.

Brown, P. (2006). Advantages and disadvantages of batch farrowing. *In Practice*. 28: 94-96.

De Rensis, F., Saleri, R., Tummaruk, P., Techakumphu, M. and Kirkwood, R. N. (2012). Prostaglandin $F2_{\alpha}$ and control of reproduction in female swine: A Review. *Theriogenology*, 77: 1-11.

Estill, C. T., Britt, J. H. and Gadsby, J. E. (1993). Repeated administration of prostaglandin $F_{2\alpha}$ during the early luteal phase causes premature luteolysis in the pig. *Journal of Biology of Reproduction*, 49: 181–185.

GraphPad Prism Version 5.0 (2009). GraphPad Software, San Diego, California, USA (www.graphpad.com).

Guthrie, H. D. and Polge, C. (1976). Luteal function and oestrus in gilts treated with a synthetic analogue of prostaglandin F-2alpha (ICI 79,939) at various times during the oestrous cycle. *Journal of Reproduction and Fertility*, 48: 423-425.

International Livestock Centre for Africa (ILCA). (1992). Utilization of feed resources in relation to nutrition and physiology. *Proceedings of the 25th International Symposium of Tropical Agricultural Research*, Tsukoba Japan, September, 24th – 25th, 92.

Kouamo, J. and Kamga-Waladjo, A.R. (2013). State-of-art in oestrus synchronisation in pigs. *Revue Africaine de Sante et de Productions Animales*, 11: 110 – 115.

Kowell, J. (1986). Some physical properties of soil at Samaru, Zaria, Nigeria. Storage of water and it's use by crop. Part 1, Physical status of soil. *Nigerian Agricultural Journal*, 5: 13-20.

McGlone, J. J. (2013). The future of pork production in the world: Towards sustainable welfare-positive systems. *Animals*, 3: 401-415.

Moriyoshi, M., Nozoki, K. and Ohtaki, T. (1997). Measurement of gestagen concentration in feces using a bovine milk progesterone quantitative test EIA kit and its application to early pregnancy diagnosis in the sow. *Jounal of Veterinary Medical Science*, 59:695–701.

National Research Council (NRC). (1998). *Nutrient Requirements of Swine*. (10th edition).National Academy Press. Washington D.C. U.S.A.

Osaro, O. M (1995). Enhancing production performance of small holder pig farming. In: Oyedokun A.O and Adeshinwa (Eds). *Pig production Workshop Training Manual*, N.A.E.R.L.S/A.B.U. Zaria Nigeria pp. 100 – 130.

Przygrodzka, E., Witek, K. J., Kaczmarek, M. M., Andronowska, A. and Ziecik, A. J. (2015). Expression of factors associated with apoptosis in the porcine corpus luteum throughout the luteal phase of the oestrous cycle and early pregnancy: Their possible involvement in acquisition of luteolytic sensitivity. *Theriogenology*, 83: 535–545.

Sanders, H., Rajamahendran, R. and Burton, B. (1994). The development of a simple fecal immunoreactive progestin assay to monitor reproductive function in swine. *Canadian Veterinary Journal*, 35:355–358.

Sawa, B. A. and Buhari, B. (2011). Temperature variability and outbreak of meningitis and measles in Zaria, Northern. Nigeria. *Research Journal of Applied Sciences, Engineering and Technology*, 3(5): 399-402.

Tur, I. (2013). General reproductive properties in pigs. *Turkish Journal of Veterinary and Animal Sciences*. 37: 1-5.

Vicente, R. E., Manuel, S. R., Anton, G. and Gustavo, A. G. C. (2011). Feed conversion rate and estimated energy balance of free grazing Iberian pigs. *Livestock Science*, 132: 152-156.

Zannoni, A., Bernardini, C., Rada, T., Ribeiro, L. A., Forni, M. and Bacci, M. L. (2007). Prostaglandin F2-alpha receptor (FPr) expression on Porcine *corpus luteum* microvascular endothelial cells (pCL-MVECs). *Reproductive Biology and Endocrinology*, 5: 31-53.