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## SERUM DYNAMICS OF SOME REPRODUCTIVE HORMONES IN POSTPARTUM ANOESTRUS FRIESIAN X BUNAJI COWS TREATED WITH TWO DOSAGE REGIMENS OF OVSYNCH PROTOCOL

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### ABSTRACT

*Fertility of the Post-partum period in dairy cattle is negatively influenced by the incidence of anoestrus. This condition is characterized by the absence of oestrus behaviour which may be due to physiologic or pathologic factors. This study therefore, was aimed to compare the serum profiles of follicle stimulating hormone (FSH), luteinising hormone (LH) and progesterone (P4) in response to the administration of two doses of gonadotrophin releasing hormone (GnRH, Lecirelin®) in combination with prostaglandin F2 alpha (PGF2α, Synchronate®) ovsynch protocol in apparently healthy, post-partum anoestrous Friesian x Bunaji cows. Fifteen (n=15) animals were randomly divided into three groups and treated as follows: Group I (n=5) animals (control); Group II (n=5) animals received ovsynch 50 µg GnRH treatment; Group III (n=5) animals received 100 µg GnRH ovsynch treatment. Blood samples were collected at 15 to 30 minutes intervals for 6 hours to determine serum concentration of FSH and LH and once daily for 12 days after the injection of the first GnRH to determinate the concentration of plasma P4 using Enzyme linked immunosorbent assay kits. Peak values of FSH and LH were significantly ( $p<0.05$ ) different on days 0 and 9. The plasma P4 levels increased among treated cows following injection of GnRH reaching a peak on day 7 compared to the control group. It was therefore concluded that the ovsynch protocol at the dosages indicated enhanced FSH, LH and P4 profiles compared to control cows and could initiate the onset of ovarian activity in the post-partum anoestrus Friesian x Bunaji cows.*

**Keywords:** Serum, Follicle Stimulating Hormone, Luteinizing Hormone, Plasma Progesterone, Friesian Bunaji Cow

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### INTRODUCTION

The annual calf crop percentage is an indication of the reproductive efficiency of any cow-calf operation (Duggan *et al.*, 2021). For cows to calve every year, it is imperative that the number of days such cows would be opened be regularly monitored. Anoestrus is a kind of infertility signifying a lack of oestrus expression at an expected time (Jena *et al.*, 2016). The appropriate manifestation of oestrus resulting from normal ovarian cyclicity is critical to successful reproductive performance in cattle (Degefa *et al.* 2016).

The administration of GnRH produces acute increases in FSH and LH in cattle and the magnitude depends on the stage of the follicular wave and circulating steroid hormone concentrations (Nett *et al.*, 2002; Colazo *et al.*, 2009). Jensen *et al.* (1983) reported that the magnitude of plasma LH response increased with the increasing dosage of GnRH in diestrous heifers with the greatest increase occurring between 50 and 100 µg. However, Kohran *et al.* (1998) showed that hormonal changes did not occur during the oestrus cycle of heifers and cattle treated with GnRH at different stages of the oestrous cycle. Therefore, the objective of the present study was to evaluate the hormonal response of post-partum anoestrous Friesian x Bunaji cows to two dosage regimens of ovsynch protocol given at different stages of the oestrous cycle.

## **MATERIALS AND METHODS**

### **Ethical Approval**

The experimental procedures were conducted in accordance with standards of the Ethics committee for animal experimentation, Ahmadu Bello University, Zaria, Nigeria and the approval No ABUCAV/2021/114.

### **Study Site**

The present study was conducted in a private farm located in Maraban Jos, Igabi Local Government Area of Kaduna State situated at a geographical coordination of 10 47' 0" North 7 46' 0" East.

### **Treatment Protocol**

Fifteen (n=15) lactating Friesian x Bunaji crossed cows weighing 200 – 250 kg and not exhibiting oestrus for 90 days or more post partum were selected and allotted randomly into three groups of five animals each. The cows were maintained on a semi-intensive method of production and treated as follows: Group 1 (n=5); control (c) All animals received 2 mL normal saline at the time of hormonal treatments.

Group II (n =5); Half dosage (HD). The animals received 2 mL of (50 µg) I.M injection of lecorelin (Biovetaa. S Komenskeho 2/2/12, Czech Republic) on day 0 followed by 2 mL (500µg) I.M injection of PGF<sub>2</sub>α analogue Cloprostenol (Estrumate<sup>®</sup>, Schring-Plough, Animal Health) on day 7 and again 2 mL (50 µg) GnRH I.M injection on day 9. Group III (n=5) full dosage (FD) all animals received as in Group II, 4 mL (100 µg) I.M injection of lecorelin on day 0, followed by 2ml (500 µg) I.M injection of synthetic PGF<sub>2</sub>α analogue Cloprostenol on day 7 and 4 mL (100 µg) I.M injection of lecorelin on day 9.

### **Collection of Blood Sample**

A 5 mL blood samples for FSH and LH assay were obtained just before the GnRH administration and at 15 min, 30 min, 0-6 hours after GnRH administration. For progesterone assay, blood samples were obtained on day before GnRH administration and on days 1 to 12 after the first GnRH administration. Blood samples were immediately chilled on ice and centrifuged (3000 x g 10 mins) within 2 h of collection. Plasma and serum samples were stored at -20°C until assayed.

### **Hormonal Analysis**

The serum FSH and LH concentrations were determined by an immune enzymometric enzyme linked immunosorbent assay kits (Accubind Elisa, Monobind Inc. 100 North Point Drive Lake Forest, CA 92630, USA). The plasma Progesterone concentration was evaluated by a competitive progesterone ELISA kit. (Accubind Elisa, Monobind Inc).

### **Statistical Analysis**

Data generated were subjected to statistical analysis by two way analysis of variance and Fisher's least significant difference LSD using Graph pad prism version 8.0 for windows, graph pad software, san - Diego California USA, [www.graphpad.com](http://www.graphpad.com). Values of P<0.05) were considered statistically significant.

## **Results**

The concentration of FSH in serum during a 360 minutes period after an injection of saline (control) or GnRH at 50 µg or 100 µg are illustrated in Figures 1 and 2. No significant increase in serum FSH was observed in the control cows during a 6 h sampling period. The concentrations of serum FSH in treated cows increased above those in control groups within 15-30 minutes and remain elevated up to 300 minutes post GnRH administrations on day 0 and day 9. The peak concentrations of FSH in treated groups on day 9 tend to be higher than those of day 0.

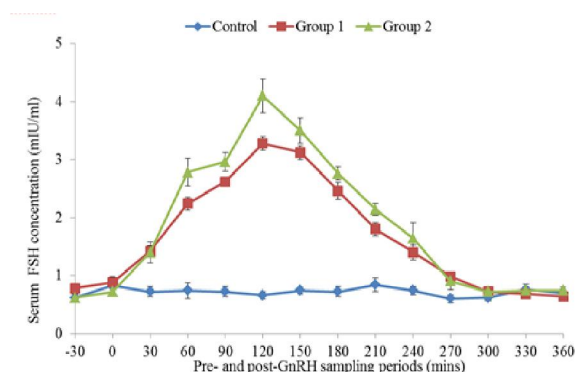


Figure 1: Mean (±SEM) serum concentrations of follicle stimulating hormone in Friesian x Bunaji cows treated with saline, 50 µg and 100 µg GnRH at day 0

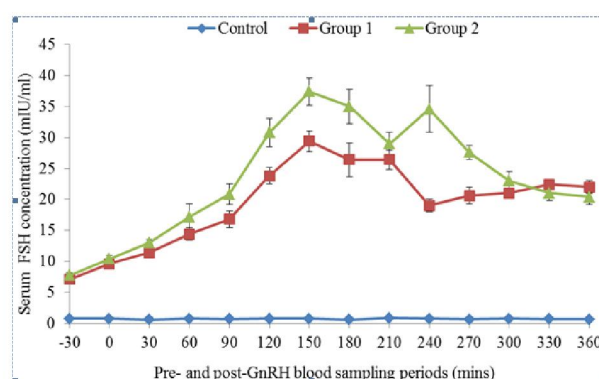


Figure 2: Mean (±SEM) serum concentrations of follicle stimulating hormone in Friesian x Bunaji cows treated with saline, 50 µg and 100 µg GnRH at day 9.

### Luteinising hormone

The average serum concentration of LH during 360 minutes period after injection of saline (control) or GnRH against at 50 g or 100 g on day 0 and day 9 are illustrated in Figures 3 and 4. There were no significant changes in the serum LH of Friesian x Bunaji cows administered with saline during the 360 minutes samples period. However, the serum LH of treated groups increased above that of the control within 30 minutes and significantly by 60 or 120 minutes after GnRH treatment and decreased to basal levels by 240 minutes. The LH Concentrations peaked 120 minutes after GnRH treatments and the peak concentration tend to be higher in cows treated with GnRH 48 hours after PGF<sub>2</sub> $\alpha$  than those treated on day 0.

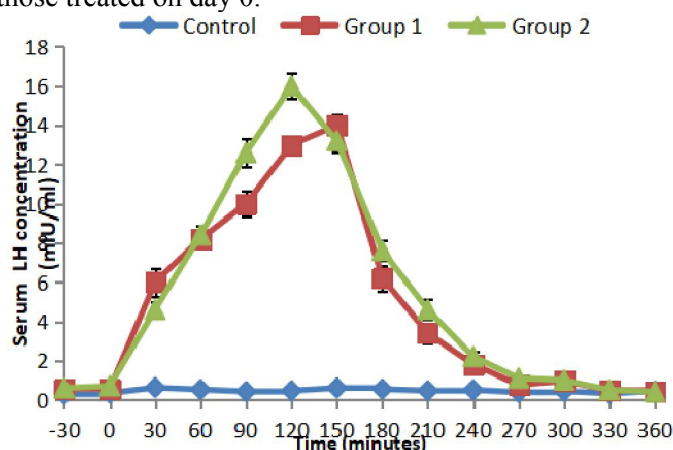


Figure 3: Mean (±SEM) serum concentrations of luteinizing hormone in Friesian x Bunaji cows (n = 5 each) administered with saline, 50 µg and 100 µg GnRH agonist at day 0.

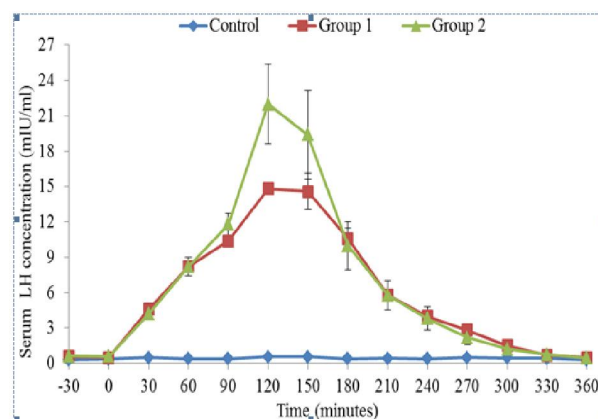
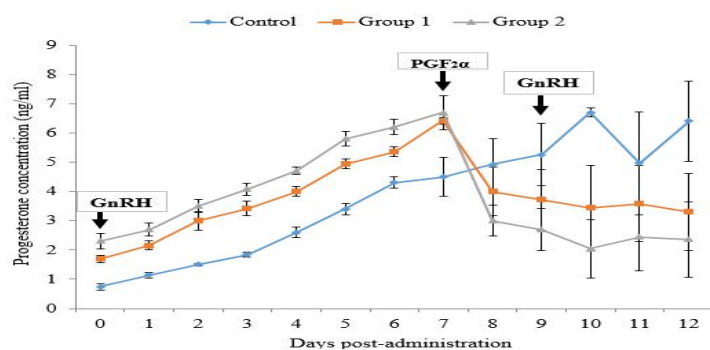


Figure 4: Mean (±SEM) serum concentrations of luteinizing hormone in Friesian x Bunaji cows (n = 5 each) administered with saline, 50 µg and 100 µg GnRH agonist at day 9.

### PLASMA PROGESTERONE

A highly significant variation in P<sub>4</sub> levels among different days of the protocol (days 0, 7, and 9) was observed in treated groups with a higher value recorded on day 7. Concentrations of P<sub>4</sub> in Plasma increased progressively after injection of GnRH and remained elevated until day 7 post injections (Figure 5). The plasma P<sub>4</sub> concentration declined from day 8, the day after PGF<sub>2</sub> $\alpha$  treatment and reached their lowest around day 11. The Plasma P<sub>4</sub> continues to rise in the control group after day 8 reaching a peak on day 12. The plasma P<sub>4</sub> also tended to peak at higher concentration in treated groups than in controls. In all the groups, plasma P<sub>4</sub> on the day of initiating treatment was low. There was no significant difference in the pattern of P<sub>4</sub> response to GnRH among treatment groups.



**Figure 5:** Mean ( $\pm$ SEM) plasma concentrations of Progesterone in Friesian x Bunaji cows ( $n = 5$  each) administered with saline, 50  $\mu$ g and 100  $\mu$ g GnRH agonist.

## DISCUSSION

The treatment of Friesian x Bunaji cows with 50  $\mu$ g and 100  $\mu$ g of leclirelin a GnRH agonist on day 0 and day 9 were able to elicit sufficient FSH and LH responses when compared to the control. This is in agreement with earlier researchers that investigated the potency of GnRH agonists (Rettmer *et al.*, 1992; Picard Hagen *et al.*, 2015). The findings of the present study suggest a higher peak concentration with the 100  $\mu$ g dosage on days 0 and 9 than the 50  $\mu$ g dosage. This tends to agree with the findings of Jensen *et al.* (1983) and partly with Colazo *et al.* (2009) who reported that plasma concentrations of LH increased with increasing doses of GnRH. The findings of the present study also negate the findings of Kohram *et al.* (1998). The findings of the present study also suggest that higher serum FSH release occurred on day 9 of GnRH administration than day 0, likewise, the serum FSH concentration failed to return to pre-treatment concentrations by 300-360 min after GnRH administration on day 9. This could be as a result of the stage of the oestrous cycle and because the newly recruited follicles are yet to diverge into dominant and subordinate follicles when the influence of FSH is expected to decline (Martinez *et al.*, 2003).

The circulating progesterone value in groups II and III increased significantly  $P < 0.05$  on day 7 when compared to the control group. This finding corroborates the observation made by earlier workers (Pursely and Marthins, 2011; Picard Hagen *et al.*, 2015). The increase in Plasma  $P_4$  could be as a result of the luteo-protective and anti luteolytic effects of the GnRH against the secretion of estradiol (Rettmer *et al.*, 1992).

## CONCLUSION

The full dosage group caused higher FSH and LH release than the half dosage group, likewise, the GnRH administration on day 9 caused higher FSH and LH releases than that of day 0. Further studies are necessary with more animal units to validate present findings.

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