FUDMA Journal of Agriculture and Agricultural Technology
ISSN: 2504-9496
Vol. 9 No. 3, September 2023: Pp. 87-93

https://doi.org/10.33003/jaat.2023.0903.13

EFFECT OF GONADOTROPHIN RELEASING HORMONE ON FOLLICULAR DYNAMICS, LUTEINISING HORMONE, AND PROGESTERONE LEVELS IN BUNAJI COWS

Dare, T. A.[1*], Mamman, M.[2], Kawu, M. U.[3], Chom, N. D.[4]

[1]College of Agriculture and Animal Science, Ahmadu Bello University, Mando, Kaduna.
[2]Department of Veterinary Pharmacology and Toxicology, Ahmadu Bello University, Samaru, Zaria
[3]Department of Veterinary Physiology, Ahmadu Bello University, Samaru – Zaria
[4] Radiology Unit, University Teaching Hospital, Ahmadu Bello University, Shika, Zaria
* Corresponding Author aderemidarefm@gmail.com https://orcid.org/0000-0002-9084-515

ABSTRACT

This study evaluated the ovarian and hormonal responses of Bunaji cows to ovsynch protocol initiated at random oestrus cycle stages. Fifteen animals were arranged into three groups and treated as follows: control (n=5), 2mL normal saline; group A (n=5), 50µg Gonadotrophin releasing hormone (GnRH) on day 0 and day 9 followed by 500µg of Prostaglandin F2 alpha ($PGF_2\alpha$) on day 7; and group B (n=5) 100µg of GnRH on day 0 and day 9 followed by 500µg $PGF_2\alpha$ on day 7. Ultrasound examination of the ovaries was carried out daily from d 1 to d 9 and, thereafter every 12h until ovulation was detected. Blood sampling was carried out at 30 min intervals for 6 h following the GnRH administration for determination of serum luteinizing hormone (LH) and once daily for 12 days after the injection of the first GnRH to determine plasma progesterone concentration. The ovarian response of the treated groups showed no significant difference between the two dosages of ovsynch protocol but differed significantly between the treated and the control groups. Peak values of serum LH obtained on day 0 and day 9 of GnRH administration differed significantly ($P<0.05$). Plasma progesterone increased among treated groups following injection of GnRH reaching a peak on day 7 compared to control, but did not differ among treated groups. In conclusion, GnRH at 50 µg and 100 µg was able to elicit adequate LH and progesterone responses that caused the disappearance of dominant follicles and, subsequent ovulation thereby increasing the reproductive efficiency of Bunaji cows.

Keywords: Bunaji Cows, Follicle, GnRH, LH, Progesterone.

INTRODUCTION

Gonadotrophin releasing hormone (GnRH) stimulates the production and release of follicle stimulating hormone (FSH) and luteinizing hormone (LH) from the anterior pituitary. The main indicators of the efficacy of treatment with GnRH include improved LH secretion, dominant follicle disappearance, and formation of a new corpus luteum (Hussien *et al*., 2021). The size and functional status of the dominant follicle and oestrus cycle phase may also affect the responses to GnRH treatment (Atkins *et al.*, 2008; Colazo *et al*., 2008). The Ovsynch protocol was developed to synchronise ovulation for fixed-time insemination (FTI) in dairy cows (Pursely *et al*., 1995) using GnRH and $PGF_2\alpha$, and is also used to manage suboestrus cows without the need for oestrus detection. The procedure consists of two injections of GnRH 9 days apart and administration of $PGF_2\alpha$ 48 h prior to the second GnRH injection. The first injection of GnRH is given at a random stage of the oestrous cycle which causes ovulation or luteinization of the dominant follicle in most animals (Hussien *et al*., 2021). The reason for the second GnRH treatment two days after the $PGF_2\alpha$ is to push back the timing of the LH surge and to synchronise ovulation such that single insemination is enough to ensure fertility (Atanasov *et al*., 2021; Randi *et al*., 2021; Pursely *et al*., 1995). However, because of multiple injections and handling administered at specific times, a greater direct cost of initial treatment is incurred. The ovsynch protocol requires three hormonal injections with the GnRH responsible for approximately 70 % of the total cost in a dairy cow (Baranski *et al*., 2021; Karaca *et al*., 2016). The cost of hormones needed to synchronise oestrus and ovulation has been one of the major challenges facing the adoption of improved breeding techniques, since reproductive intervention has to be cost effective (Fricke, 1999). There is a dearth of information on appropriate GnRH dosage to induce oestrus and synchronize ovulation in Bunaji cows. Thus, we investigated the effect of two GnRH doses (full and half-full) on ovulation synchronisation, LH, and progesterone profiles in Bunaji cows.

MATERIALS AND METHODS

The study was carried out in the Livestock section of the Samaru College of Agriculture, Division of Agricultural Colleges, Ahmadu Bello University, Zaria, situated in the Northern guinea savannah zone of Nigeria, between latitudes 11^0 and 12^0N and longitudes 7^0 and 8^0E at an elevation of 650 m above sea level. The average annual maximum and minimum temperature are between 31.0±3.2 and 18.0±3.7^{0}C. The average annual rainfall is 1100 mm lasting from May to October with a mean relative humidity of 72%. The dry season lasts from November to April with mean daily temperature ranging from 15^0C - 36^0C and mean relative humidity 20% - 37% (Rekwot *et al*., 1998).

Experimental Animal Management

The study was conducted with fifteen apparently healthy and cycling Bunaji cows aged between 3 to 8 years (4.87±1.85 years). The animals were managed semi intensively with the provision of supplementary feeding, roofed housing, veterinary care, and record keeping. The animals were individually identified with large plastic ear tags and screened for blood and gastrointestinal parasites.

Routine treatments and vaccination against prevalent diseases were carried out before the commencement of the study. The mean body condition score of the animals was 3.5±0.65. The general characteristics of the animals used are shown in Table 1. Ethical approval for the experiment was obtained from the Committee on Animal Use and Care, Ahmadu Bello University, Zaria with approval number ABUCAU/2021/114.

Table 1: Reproduction and Production Conditions of the Experimental Animals.

Parameter	**(Bunaji cows)**
Time of Experiment	August
Pluripara	n =15
Average daily milking yield (kg)	2 kg
Body condition score	3.0 ± 0.65
Presence of corpus luteum on ovary/cycling	Present/cycling
Mean Age (years)	4.53 ± 1.96

Experimental Design

Fifteen non-lactating apparently healthy and cycling Bunaji cows (n=15) at different stages of the oestrous cycle were allotted randomly to three groups of five animals each and treated as follows: Group 1 (n=5), received 2mL normal saline at the time of hormonal treatments; Group 2 (n=5), received 50 µg IM injection of a synthetic GnRH analogue, LECIRELIN® (Bioveta,a.s. Komenskeho 212/12, Czech Republic) on days 0 and 9, and 500 µg IM injection of a synthetic $PGF_2\alpha$, Clorprostenol (SYNCHROMATE®; Bremer Pharma B.V., Germany) on day 7. Group 3 (n=5), was treated with 100µg IM injection of LECIRELIN® on days 0 and 9 and 500 µg IM injection of $PGF_2\alpha$ on day 7.

Blood sampling

Blood samples were collected by jugular venipuncture and shared into a set of EDTA test tubes for plasma progesterone assay and a set of plain test tubes for the determination of serum LH. The first blood sample for LH assay was collected just prior to the GnRH administration, while subsequent samplings were done at 15 min, 30 min, 1 h, 2 h, 3 h, 4 h, 5 h, and 6 h after the administration of GnRH. While, the first blood sampling for progesterone assay was done a day before GnRH administration, thereafter, on a daily basis from day 1 to day 12 after the first GnRH treatment. The blood samples were then placed in ice packs, taken to the lab, and centrifuged at 3000 x g for 10 mins within 2 h of collection. The plasma and serum samples were kept at -20°c until analysed.

Ultrasonography

A trans-rectal ultrasonographic examination of the ovaries was performed a day before GnRH administration, on a daily basis until the second GnRH, and thereafter, at every 12 h until ovulation occurred. The ovarian ultrasonography was done using a real-time B-mode scanner (DP, 2200 Vet Mindray Co. Ltd. Shenzen, China) equipped with a 5.0 MHtz linear array rectal transducer. During the ultrasonography, the location of the largest follicle was recorded and the follicles were classified into i) small (<0.5 cm), ii) medium (0.5-1.0 cm), and iii) large (>1.0 cm) based on diameter size. These classifications were used to determine the day of emergence of follicle wave, day of follicle deviation (Ginther *et al*., 1997), the size of the follicle at the time of deviation of the future ovulatory follicle, day of ovulation (the day when the ovulatory follicle disappeared from the ovary between two consecutive ultrasound examinations), number of ovulations, size of ovulatory follicle and, presence and size of CL on each day. Ovulation was confirmed by the detection of growing CL 3 to 5 days after ovulation. The images of relevant ovarian structures were captured and recorded on the monitor, while measurements were taken with the in-built caliper system. All the ultrasonographic procedures were carried out by one operator.

Hormone Analysis

Luteinising Hormone Assay

The serum LH concentration was determined using an LH ELISA Kit (Accubind ® ELISA, Monobind Inc. 100 North Pointe Drive, Lake Forest CA92630, USA). The assay sensitivity was 0.003 m/u/well. While, the mean intra-and inter-assay coefficients of variation were 6.8%, 3.9%, and 3.6%; and 7.8%, 10.8%, and 9.6%, respectively.

The Progesterone Hormone Assay

The determination of plasma progesterone concentration was done using a Progesterone ELISA Kit (Accubind ® ELISA, Monobind Inc. 100 North Pointe Drive, Lake Forest CA92630, USA). The assay sensitivity was 0.105ng/ml. While, the mean intra-and inter-assay coefficients of variation were 15.3%, 3.6%, and 6.1%; and 8.9%, 7.5%, and 6.4%, respectively.

Statistical Analysis

Data was presented as mean (±SEM), while descriptive statistics and tests of associations were used to analyse the data. The effect of time after ovulation on various parameters was assessed by one-way ANOVA. Mean values were compared using two-way repeated measures ANOVA and Fisher's least significant difference LSD. All tests were performed using the GraphPad Software, San Diego California USA, www.graphpad.com. Values of $P<0.05$ were considered statistically significant.

RESULTS

Table 2 depicts the results of Bunaji cows that ovulated following the first and second GnRH analogue injections and other parameters associated with ovarian follicular dynamics. The control group had 0% and 20% ovulation rates at the time of the first and second GnRH administrations, respectively. There was a significant difference ($P<0.05$.) in the ovulation rates recorded for the control and treatment groups at the time of the first and second GnRH treatments. Ovsynch dosages at different periods of GnRH injection did not affect the size

of the follicles. The peak size of subordinate follicles did not differ across the groups (Table 3).

Table 2.Summary of ovarian follicular dynamics as determined by ultrasonography in Bunaji cows subjected to ovsynch synchronization protocol.

ITEM	GROUP 1	GROUP 1	GROUP 1
Day of wave emergence (Synchronized follicle)	0.00±0.00[a]	1.40±0.60[b]	1.60±0.68[b]
Wave emergence (% cows)	0[a]	40[b]	60[c]
Ovulation rate at 1[st]GnRH (%)	0[a]	40[b]	60[c]
Ovulation rate after 2[nd]GnRH (%)	20[a]	60[b]	60[c]
Day by which ovulation had occurred Post 2[nd] GnRH injection	0.40±0.40[a]	1.60±0.68[b]	1.40±0.60[b]

Values with different superscript alphabets along the same row are statistically different at P<0.05.

Table 3: Diameter of the Dominant and Subordinate Follicles in Bunaji Cows Subjected to Ovsynch Protocol

	Bunaji Cows		
	Group 1	**Group 2**	**Group 3**
Size of the dominant follicle at 1[st] GnRH Injection	8.60±1.07	10.00±1.30	11.4±1.63
Size of the pre-ovulatory follicle at 2[nd] GnRH injection (mm)	14.20±0.66[a]	11.40±1.03[b]	10.80±1.39[b]
Rate of pre-ovulatory follicle growth from Emergence to 2[nd] GnRH injection (mm/day)	0.86±0.16[a]	0.71±0,09[a]	0.83±0.13[a]
The peak size of the pre-ovulatory follicle after 2[nd]GnRH injection (mm)	14.40±0.81[a]	14.80±1.39[a]	13.60±1.50[a]
Diameter of the largest subordinate follicle at ovulation (mm)	11.40±0.60	12.80±1.39	11.40±1.47

Serum LH Concentration

There was no significant difference P>0.05 in the mean serum LH concentrations between the control and treated groups during the 6 h sampling period. However, serum LH levels in treated cows rose higher than in controls within 30 minutes; and significantly by 1 h and 2 h after GnRH treatment and then declined to basal level by 4 h. The peak levels of LH were higher in cows treated with GnRH 48 h after $PGF_2\alpha$ than those treated on day 0. The LH concentrations peaked 2 h after GnRH treatments (Figures 1 and 2).

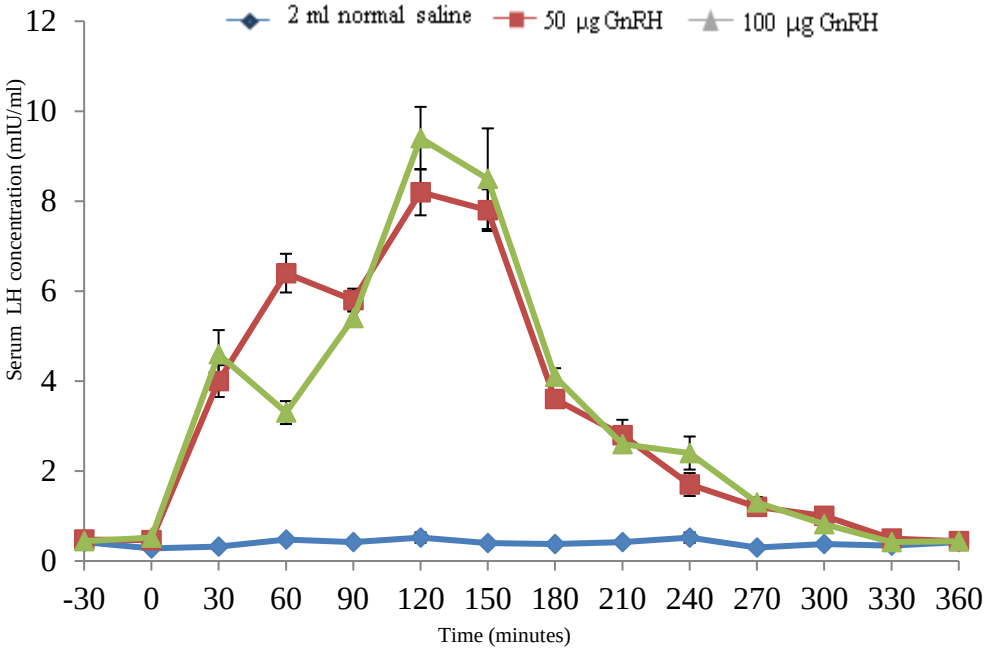

Figure 1: Mean (±SEM) serum concentrations of Luteinising hormone in control and treated Bunaji cows (n = 5 each) administered with saline, 50 µg, and 100 µg GnRH at day 0 of the experiment.

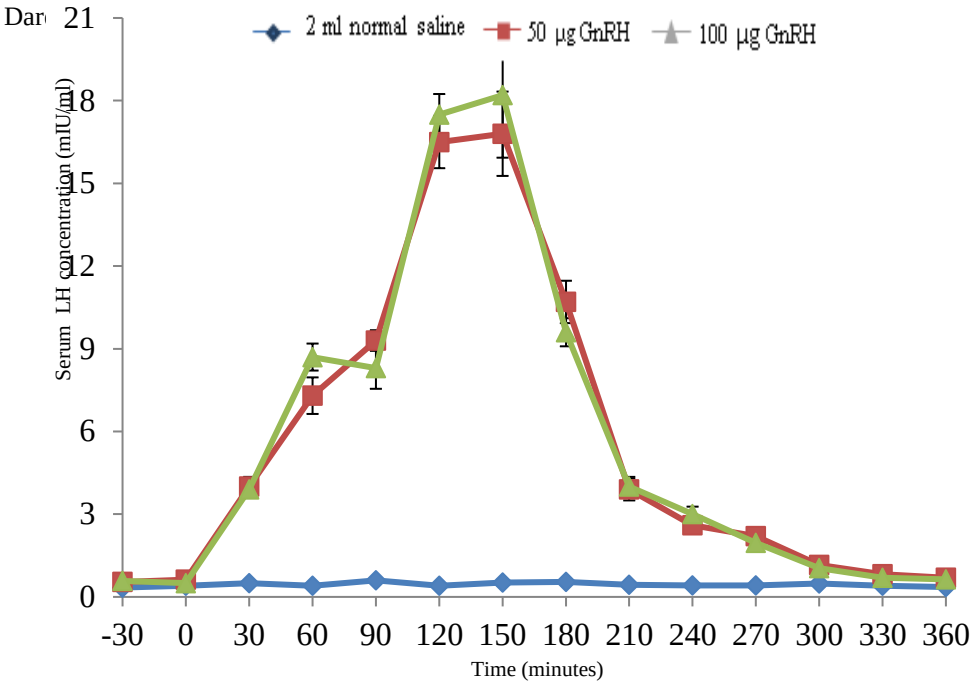

Figure 2: Mean (±SEM) serum concentrations of luteinising hormone in Bunaji cows (n = 5 each) administered with saline, 50 µg, and 100 µg GnRH agonist at day 9 of the experiment.

Plasma Progesterone

A significant difference in the Progesterone (P_4) concentrations was observed between days 0, 7, and 9 in both 50 µg and 100 µg dose groups with a higher value recorded on day 7. The concentration of P_4 in plasma increased progressively after injection of GnRH and remained elevated until day 7 post injections. The Plasma P_4 concentration declined from day 8, the day after $PGF_2\alpha$ treatment, and reached its lowest around day 11. Plasma P_4 continues to rise in the control after d 8 reaching a peak on d12. The Plasma P_4 also tended to peak at higher concentrations in treated groups than in control. Plasma P_4 concentration on the day of initiating treatment was low (Figure 3).

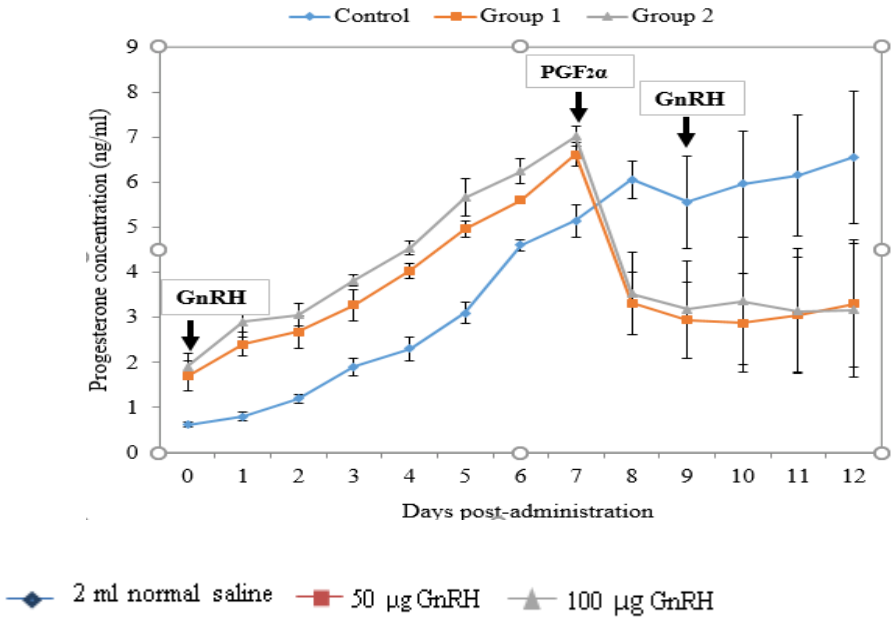

Figure 3: Mean (±SEM) plasma concentrations of Progesterone in Bunaji cows (n = 5 each) treated with 2 ml normal saline, 50 µg and 100 µg GnRH agonist.

DISCUSSION

The ovsynch protocol adjusts ovarian follicular function to allow for AI at a predetermined period, reduced handling of the animals, and improved time management for ovulation synchronization and AI (Meglic *et al.*, 2023). The mean diameter of ovulatory follicles after the second GnRH administration was not different between the 50 µg and 100 µg dosage groups in this study. This finding agrees with an earlier report by Ahmadzaden *et al.*, 2007 who observed that ovulatory follicle size in lactating cows treated with 100 µg or 50 µg of GnRH was not different between the two dose groups. Our study recorded an ovulation rate of 60% in both the 50 µg and 100 µg dose groups following the second GnRH administration. This finding was lower than those obtained by Picard Hagen *et al.* (2015), Ahmazaden *et al.* (2007), and Yamada *et al.* (2002) who reported ovulation rates of 81% to 100% after the second GnRH treatment with the full and half-full doses of GnRH. Environmental conditions, nutritional status, and source of GnRH and

$PGF_2\alpha$ have been reported to affect reproductive outcomes following ovsynch protocol in dairy cows (Hussien *et al.,* 2021). The treatment of Bunaji cows with the 50 μg and 100 μg GnRH agonist on day 0 and day 9 was able to elicit sufficient LH and P_4 responses to ensure that the dominant follicle in most of the cows disappeared and new follicular wave development is initiated. This is in agreement with earlier reports that investigated the potency of GnRH agonists (Picard-Hagen *et al.,* 2015; Martinez *et al*., 2003; Rettmerer *et al*., 1992). The efficacy of the GnRH agonists has been attributed to their low affinity to degradation by pituitary endopeptidases (Lasdun and Orlowski, 1990). The findings from this study also suggest that higher serum LH occurred on day 9 of GnRH administration than on day 0. Several factors have been attributed to the magnitude of GnRH induced LH release. These include the stage of the follicular wave (Kastelic and Mapletoft, 1998) and circulating steroid hormone concentration (Nett *et al.,* 2002; Bleach *et al*., 2001). The decrease in LH levels in the treated groups on day 0 and the increased concentrations on day 9 may have been caused by the high and low oestradiol (E_2) levels, respectively recorded on those days. A low level of E_2 suppresses LH release; however, as E_2 increases during the follicular phase of the oestrus cycle, following a decrease in P_4 due to luteolysis, the pituitary becomes sensitized resulting in increased LH release (Breda and Kozicki, 2015; Aerts and Bols, 2010). On the other hand, high levels of P_4 produced by a functional corpus luteum in diestrous or pregnancy decrease the pulsatile LH response to GnRH (Breda and Kozicki, 2015).

The higher plasma P_4 concentration following the first GnRH treatment up to day 7 in the treated cows as compared to control cows agrees with the reports of Picard-Hagen *et al.* (2015) and Pursely and Martins (2011), who reported elevated plasma concentrations of P_4 following GnRH treatment. The increase in plasma P_4 could be viewed as evidence of the luteo-protective and anti-luteolytic effects of the GnRH (Rettmer *et al*., 1992). It could also be attributed to the development of an accessory CL caused by the acute increase in LH following the GnRH treatment or increased growth of luteal cells in the accessory CL (Davis *et al*., 2003; Twagiramungu *et al*., 1995). The $PGF_2\alpha$ given on day 7 intended to lyse the previous CL, resulting in new oestrus and ovulation, caused rapid decline in P_4 concentrations within 24 h of the $PGF_2\alpha$ treatment in 60% of the treated cows, indicating complete luteolysis in the affected animals. This agrees with previous findings (Stevenson, 2016; Ali and Fahmy, 2007) that if a CL resulted from the first GnRH treatment, the 7-day interval is adequate for it to mature and be responsive to $PGF_2\alpha$ treatment. Furthermore, incomplete or lack of regression of the CL could have been responsible for the 40% of cows that did not respond to $PGF_2\alpha$ treatment. These findings are in agreement with those of Pinheito *et al.* (1998) and Rekwot *et al.* (1999) who reported that incomplete Cl regression and increased P_4 levels during the follicular phase could limit oestrus activity and subsequent ovulation.

CONCLUSION

In conclusion, the findings of this study suggest that the ovsynch protocol with 50 μg GnRH (half dose) is as effective as the 100 μg GnRH (full dose) in inducing LH and P_4 responses, and synchronization of new follicular wave in Bunaji cows. Further studies are recommended with a larger sample size of cycling Bunaji cows to validate these findings.

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