EFFECT OF GONADOTROPHIN RELEASING HORMONE ON LUTEINIZING HORMONE SURGE, OVULATION AND PROGESTERONE LEVELS IN BUNAJI COWS.

T.A. Dare[1*], M. Mamman[2], M.U. Kawu[3], N.D. Chom[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 (E-mail: theophilusdare@yahoo.com)

ABSTRACT
This study evaluated the ovarian and hormonal responses of Bunaji cows to ovsynch protocol initiated at a random stage of oestrus cycle. Animals were arranged into three groups; control (n=5), groups A (n=5) and B (n=5). The control group received 2mL normal saline at time of hormonal treatments. Group A received 50μg GnRH on day 0 and day 9followed by 500μg of $PGF_2\alpha$ on day 7. Group B received 100μg of GnRH on day 0 and day 9 followed by 500μg $PGF_2\alpha$ on day 7. Ultrasound examination was done from day 1 to 9 and after every 12h until detection of ovulation. Blood was sampled at 15 to 30 minutes intervals for 6 h after the GnRH administration to determine serum concentration of LH and once daily for 12 days after the injection of the first GnRH to determine concentration of plasma progesterone. 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 differs significantly ($P<0.05$). Plasma progesterone increased among treated groups following injection of GnRH reaching a peak on day 7 than in control, but did not differ among treated groups. In conclusion, GnRH analogue at the doses indicated were able to elicit sufficient LH and progesterone responses to cause 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) is a releasing hormone responsible for the release of follicle stimulating hormone (FSH) and luteinizing hormone (LH) from the anterior pituitary. It is a decapeptide hormone synthesized and released from GnRH neurons in the basal hypothalamus and a key regulator of the mammalian reproductive system (Picard-Hagen *et al.,* 2015; Zerani *et al.,* 2021). For any of the clinical or management uses of GnRH analogues, the key biological responses to evaluate the efficiency are an adequate LH surge, the disappearance of the dominant follicle and the formation of accessory corpus luteum. However, the size and functional state of the dominant follicle at the time of GnRH administration and the phase of the oestrus cycle may be critical determinants of the responses to GnRH treatments (Atkins *et al.,* 2008).The cost of hormones needed to synchronize oestrus and ovulation has been one of the major challenges facing adoption of improved breeding techniques as reproductive intervention has to be cost effective (Fricke, 1999). There is a dearth of information on appropriate GnRH dosage to induce oestrus and ovulation sychronozation in Bunaji cows. Thus, we investigated the effect of GnRH at two different doses on ovulation, LH surge and progesterone in Bunaji cows.

Materials and Methods
Fifteen apparently healthy, cycling, and pluriparous Bunaji cows from the Livestock Section, Samaru College of Agriculture, ABU, Zaria were utilized for the present study (Ethical approval, Animal Use and Care, ABUCAU/2021/114). Animals were randomly arranged into control group C (n=5), administered 2 mL of normal saline, while treated groups A (n=5) received 50μg GnRH I.M (Lecirelin® Bioveta, a.s. Komenskeho, 212/12, Czech Republic) on day 0, 500μg of $PGF_2\alpha$ on day 7 and the second administration of 50μg of GnRH on day 9 and treated group B received 100μg of GNRH on day 0, 500μg $PGF_2\alpha$ and a second dose of 100μg GnRH on day 9. Daily ultrasound examinations were performed using trans-rectal ultrasonography connected to a 5.0 mHz transducer (Dp-2200 Vet Mindray Co. Ltd, China). Ovaries were scanned in several places to identify all the

follicles greater than 4 mm from day -1 to day 9, thereafter daily to detect ovulation. Desired images were frozen on the screen and the measurements were taken. The corpus luteum (CL) was present on the ovary in all the cows before treatment. Blood samples were collected from jugular vein into two test tubes one containing EDTA for plasma progesterone assay and the other tube containing no anticoagulant for serum preparation for LH hormonal assay. Blood samples for LH concentration were obtained just before the GnRH administrations and at 15, 30 minutes 1, 2, 3, 4, 5, 6 h after GnRH administration. Blood samples for progesterone concentration were obtained on the day before GnRH administration and at days 1 to 12 after the first GnRH administration. Blood samples were immediately chilled on ice and centrifuged (3000 xg 10minutes) within 2 h of collection, plasma and serum samples were stored at -20 until assayed. The serum LH and Plasma Progesterone Concentration (PPC) were determined by immune enzymometric ELISA Kit (Accubind® ELISA, Monobind Inc, USA). For LH, the sensitivity of the assay was 0.003m/u/well and the mean intra-assay coefficients of verification for levels 1, 2 and 3 pooled control sera were 6.8%, 3.9% and 3.6%, respectively. The inter-assay precision coefficients of variation for levels 1, 2 and 3 pooled control sera were 7.8%, 10.8% and 9.6%, respectively. For PPC, the sensitivity of the assay was 0.105ng/ml. The mean intra-assay coefficients of variation for the low, normal and high pooled control sera were 15.3%, 3.6% and 6.1%, respectively. The inter-assay precision coefficients of variation for the low, normal and high pooled control sera were 8.9%, 7.5% and 6.4%, respectively.

Statistical Analysis

Descriptive Statistics and test of associations were used and the data was presented as the mean±standard error of mean (SEM). 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 Graph and Software, San Diego California USA, www.graphaand.com. Values of $P<0.05$ were considered statistically significant.

Results

Table 1 depicts results of Bunaji cows that ovulated following first and second GnRh analogue injections and other parameters associated with ovarian follicular dynamics. Control group had 0% and 20% ovulation rate at the time of first and second GnRH ($P<0.05$) administrations, respectively. There was a significant difference in the ovulation rates recorded for the control and treatment groups at the time of first and second GnRH treatment. Ovsynch dosages at different periods of GnRH injection did not affect the size of the follicles. The peak size of surbordinate follicles did not defer across the groups.

TABLE 1.Summary of ovarian follicular dynamics as determined by ultrasongraphy in Bunaji cows subjected to ovsynch synchronization protocol.

ITEM	GROUP 1	GROUP 2	GROUP 3
Day of wave emergence	0.00 ± 0.00^{a}	1.40 ± 0.60^{b}	1.60 ± 0.68^{b}
Synchronized follicle			
Wave emergence (% cows)	0	40	60
Size of dominant follicle at 1st GnRH administration	8.6 ± 1.07^{a}	11.4 ± 1.03^{b}	10.8 ± 1.39^{b}
Ovulation rate at 1st GnRH (%)	0^{a}	40^{b}	60^{c}
Ovulation rate after 2nd GnRH (%)	20^{a}	60^{b}	60^{b}
Maziimum size of ovulatory follicle (mm)	14.40 ± 081^{a}	14.80 ± 1.39^{a}	13.60 ± 1.50^{a}
Peak size of subordinate follicle (mm)	$11.400.60^{a}$	$12.801.39^{a}$	$11.401.47^{a}$

Values with same superscript alphabets along the same row are not statistically different at $P<0.05$.

Serum LH Concentration

No significant changes in serum LH were observed in Bunaji cows administered with Saline during the 6 h sampling period. Serum LH increased above that of control within 30 minutes and significantly by 1 or 2 h after GnRH treatment and decreased to basal level by 4h. Peak concentrations

of LH tend to be 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).

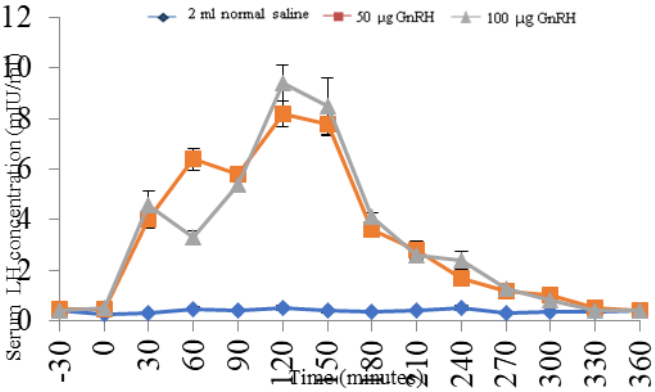


Figure 1: Mean (±SEM) serum concentrations of Luteinising hormone in control and treated Bunaji cows (n = 5 each) administered with saline, 50 µg and 100 µg GnRH at day 0 of experiment.

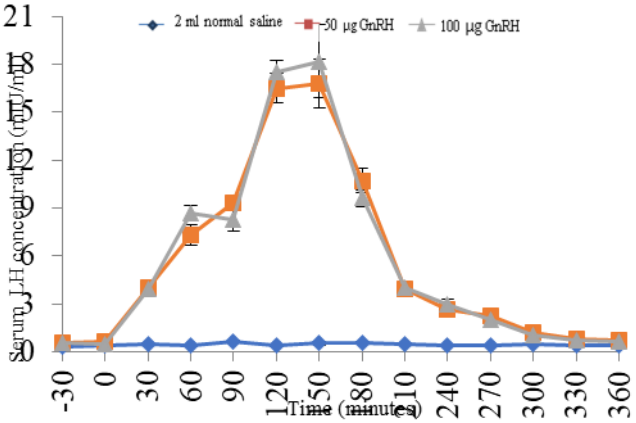


Figure 2: Mean (±SEM) serum concentrations of luteinising hormone in Bunaji cows (n = 5 each) administered with saline, 50 µg and 100 µg GnRH agonist at day 9 of experiment.

Plasma Progesterone

A highly significant variation in Progesterone (P_4) levels among different days of the protocol (days 0, 7, and 9) was observed in both 50 µg and 100 µg dosages with a higher value recorded on day 7. 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 their lowest around day 11. Plasma P_4 continue 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 at the day of initiating treatment was low (Figure 3).

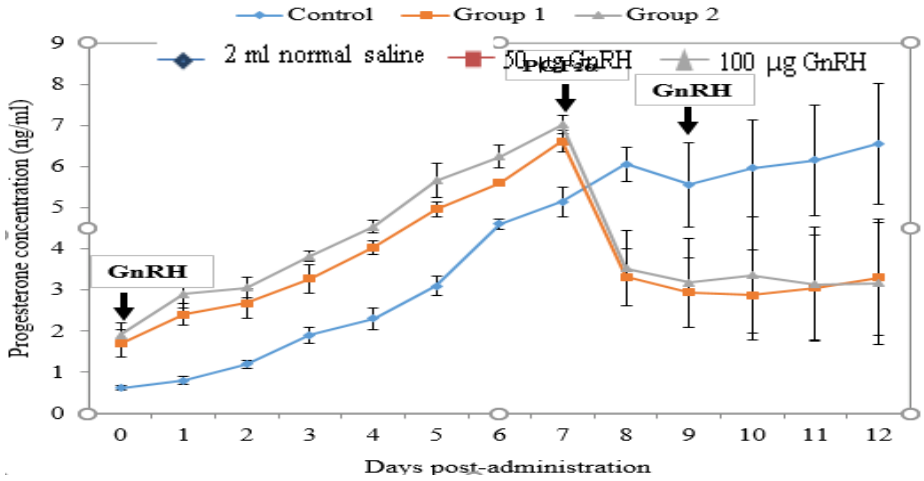


Figure 3: Mean (±SEM) plasma concentrations of Progesterone in Bunaji cows (n = 5 each) treated with 2 ml normal saline, 50 µg and 100 µg GnRH agonist.

Discussion

Ovsynch is one of the most popular hormonal protocols that controls ovarian follicular function, allows Artificial insemination at a predetermined time, which calls for less frequent handling of animals and improved time efficiency (Meglic*et al.*, 2023). In the present study, the mean ovulatory follicle diameters after second GnRH administration were not different in the 50µg and 100µg dosages of GnRH. This agrees with earlier studies (Ahmadzaden *et al.*, 2007) who reported that the size of ovulatory follicle in lactating cows treated with a full (100µg Cystorelin) or half dose (50µg Cystorelin) of GnRH in the Ovysnch protocol did not differ between full and half dose groups. Our

results revealed that ovulation rate of 60% were recorded in the 50μg and 100μg dosage groups following the second GnRH administration. These findings are lower than those obtained by Picard Hagen *et al.* (2015) and Ahmazaden *et al.* (2007), who found that the second GnRH induced ovulation in 81.8-100% dairy cows treated with Ovsynch protocols using full and half dose GnRH. Environmental conditions, nutritional status and source for GnRH and FGF2α have been reported to affect reproductive outcomes following ovsynch protocol in dairy cows (Hussein *et al.,* 2021).The treatment of Bunaji cows with the 50μg and 100μg GnRH agonist on day 0 and day 9 were able to elicit sufficient LH and P_4 responses to guarantee the disappearance of the dominant follicle in most cows and initiate the emergence of a new follicular wave. This is in complete agreement with earlier reports that investigated the potency of GnRH agonist (Picard-Hagen *et al.* 2015). The increased potency of the GnRH agonists has been ascribed to their reduced susceptibility to degradation by Pituitary endopeptidases (Lasdun and Orlowski, 1990). The findings from this study also suggest that higher serum LH release occurred on day 9 of GnRH administration than day 0. Several factors have been attributed to the magnitude of GnRH induced release of LH. These include the stage of the follicular wave and circulating steroid hormone concentration (Nette *et al.*, 2002). The higher plasma progesterone concentration till day 7 after GnRh treatment in the treated cows than those in the control agrees with those of Picard-Hagen *et al.* (2015), who reported elevated plasma concentrations of P_4 following GnRH treatment. The $PGF_2\alpha$ given on day 7 to lyse all CL and result in a new oestrus and ovulation caused a rapid decline in P_4 concentrations within 24 h of $PGF_2\alpha$ treatment in 60% of treated cows, indicating complete luteolysis in the affected animals. This agrees with previous findings (Ali and Fahmy, 2007) that if a CL resulted from the initial injection of GnRH, the 7 day interval should have provided sufficient time for the CL to mature in order to respond to $PGF_2\alpha$.

In conclusion, the present experiment suggests that the 50 μg GnRH dosage is equally effective as the 100μg GnRH dosage to elicit LH and P_4 responses to cause ovulation in most Bunaji cows and synchronization of a new follicular wave, a finding which needs to be confirmed in further study on a bigger number of cycling Bunaji cows.

REFERENCES

Ahmadzaden, A., Manzo, R., Sellars, C.B. and Dalton, J.C. (2007).The Efficacy of a reduced dose gonadrophin releasing hormone on the time and incidence of Ovulation in Holstein Cows. *Journal of Animal and Veterinary Advances,* Vo.16 Pp. 1328-1332.

Ali, A. and Fahmy, S. (2007). Ovarian dynamics and milk progesterone concentrations in cyclic and non-cyclic buffalo-cows (*Bubalusbubalis*) during Ovsynchprogra.*Theriogenology,* 68: 23-28.

Atkins, J.A., Busch, D.C., Bader, j.F., Keisler, D.H., Patterson, D.J. and Luck M.C. (2008).Gonadotrophin-Releasing Hormon-Induced Ovulation and Luteinising Hormone release in beef heifers, effect of day cycle. *Journal of Animal Science,* 89:83-93.

Fricke, P.M. (1999). New Information on timed breeding Protocols for Lactating Dairy Cows. *Advances in Dairy Technology,* 11:121-133.

Hussein, A., Sharaqy, H., Lenis, Y.Y., Jaimes, D., Turna, O. (2021). The Impact of Different Estrus Synchronization Programs on Postpartum Holstein Dairy Cow Reproductive Performance. *Mansoura Veterinary Medical Journal,* 21; 3:124-130.

Lasdun, A. and Orlowski, M. (1990). Inhibition of Endopeptidase 24.15 Greatly Increases the Release of Luteinizing Hormone and Follicle Stimulating Hormone in Response to Luteinizing Hormone/Releasing Hormone. *Journal of Pharmacology and Experimental Therapeutics,* 253:1265.

Meglic, P., Spoljaric, B., Stibric, G., Samardizija, M., Lojkic, M. et al. (2023).Ovsynch based Protocols in Reproductive Management and Infertility Treatment in Dairy Cows-hen and why?.*VeterinarskaStanica*54(2), 213-222.

Nett, T.M., Turzillo, A.M., Baratta, M. and Rispoli, L.A., (2002). Pituitary Effects of Steroid Hormones on Secretion of Follicle – Stimulating Hormone and Luteinising Hormone.*Domestic Animal Endocrinology Journal.*23:33-42.

Picard-Hogen, N, Lhermie, G., Florentin, S., Merlern, D., Frein, P. and Gayrad, V. (2015.Effect of gonadorelin, Lecirelin and Buserelin on LH Surge, Ovulation and Progesterone in Cattle.Theriogenology, Vol.84:177-183.

Zerani, M., Polisca, A., Boitic, C. and Maranesi, M. (2021). Current Knowledge on the Multifactorial regulation of Corpora Lutea Lifespan.*The Rabbit Model Animals,* 11, 296.