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OVARIAN FOLLICULAR DYNAMICS AFTER SYNCHRONISATION OF OVULATION IN BUNAJI CROSS-BRED COWS IN NORTHERN NIGERIA

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ABSTRACT

Gonadotrophin- releasing hormone (GnRH) and its synthetic analogues (Buserelin, Guserelin, Leptorelin etc) are widely used in veterinary practice to treat cystic ovarian disease and to synchronise oestrus and ovulation in cattle. The aim of this study was to evaluate the follicular dynamics in response to administration of GnRH in combination with prostaglandin F₂ alpha (PGF₂α) in treated and untreated apparently healthy Bunaji Cross-bred cows. Animals were arranged into control (n=5) and treated (n=5) groups. The treated group was given first dose of GnRH on day 0 followed by PGF₂α on day 7 and second dose of GnRH on day 9. In the control group, animals received 2 ml normal saline at time of hormonal treatments. All animals were allowed a daily ultrasound examination from day-1 to day 12. The animals were examined 12 hourly from day 9 until detection of ovulation. All animals had follicles > 8mm at time of first GnRH administration. Ovulation rates obtained in the untreated group were 0 and 20% as at the time of first and second GnRH administration respectively. This differed significantly from what obtained in the treated group where ovulation rates were 60% and 80% as at the time of first and second GnRH treatment respectively. There was no significant difference in the size of dominant follicle at time of first GnRH analogue injection and peak size of pre-ovulatory follicle after second GnRH administration. In conclusion, the ovsynch protocol has a beneficial effect on the ovulation rate when applied at any stage of the oestrus cycle in Bunaji cross-bred cows.

KEYWORDS: Bunaji Cross-bred cows, GnRH, PGF₂α Follicular Dynamic Ultrasonography

INTRODUCTION

The study of ovarian follicular dynamics and other changes in the bovine reproductive tract has progressed significantly over the past two decades, primarily due to the use of non-invasive investigative approach such as the Ultrasound (Jose and Luize, 2015). The study has opened doors to new areas of research and validated or refuted data from past reports on oestrus and ovulation synchronisations which have led to the development of improved protocols for the manipulation and control of the bovine oestrous cycle (Rajamahendra *et al.*, 2001).

In routine clinical practice of commercial dairy farms, ultrasound scanning has been reported to be an accurate and reliable method of observing dynamic changes in ovarian structures and determine the patterns of post-partum resumption of ovarian activity, silent oestrus, anoestrus and cystic ovarian conditions, and thus in reducing the calving interval (Rajamahendra and Taylor, 1990). Unfortunately, there are few studies on the use of trans-rectal ultrasonography as a diagnostic tool for reproductive disorders in Nigeria. Research-grade ultrasound machines are relatively expensive and they require a cart and external power source, making it cumbersome to operate under field conditions. The smaller battery operated machines lack the image quality of the large console base units. Therefore, the study was aimed to evaluate follicular dynamics following ovulation synchronisation within apparently healthy Bunaji cross-bred cows. The understanding of the fundamental development of antral follicles following administration of GnRH in Bunaji cross-bred cows would update current knowledge about folliculogenesis and fertility potentials of this breed in Nigeria.

**MATERIALS AND METHODS**

The study was carried out at an integrated private farm located in Maraban Jos, Kaduna (Latitude 10° 31' 32" and longitude 7° 26' 25" of Nigeria; 650 m above sea level), the average annual maximum and minimum temperatures were 31.0±3.2 and 18.0±3.70 respectively. The average annual rainfall is 11.00 mm lasting from May to October with a mean relative humidity of 72% (Osuhor, 2007). Ten (n = 10) Friesian x Bunaji Cross-bred cows were used for this study. All animals were examined ultrasonographically before the start of the treatment for identifying the state of ovulation and cyclicity. Animals were randomly allocated into two groups for control and treatment, each of which was 5 cows. In the control group all animals were injected with 2 ml normal saline comparable to the dose and time of the hormonal treatment. . Animals in the treated group (n = 5) were treated with the ovsynch protocol according to Pursely *et al.*, (1995); all animals received 100 µg 1.m injection of Lecirelin® as GnRH analogue (Bioveta, a. s., Komenskeho, 212/ 12, Czech Republic) on day 0 followed by 500 µg i.m injection of synthetic PGF₂α; Cloprostenol (Estrumate® Schering – Plough Animal Health, equal 2ml) on day 7 then by second 1.m injection of 100g GnRH analogue on day 9.

By using trans-rectal ultrasonography connected to a 5.0MHZ transducer (DP-2200 Vet, Mindray Co. Ltd, China), Ovarian morphology was monitored daily starting from the day before (day – 1), the first (day 0) to the second GnRH injection (day 9). Thereafter ovarian morphology was monitored twice daily at 12 h interval till occurrence of ovulation. The changes in follicle dynamics after the first and second GnRH treatments were analyzed. The observed follicles were classified based on diameter as measured using the internal electronic calipers. Follicle sizes were categorized as small (< 5 mm), medium (5-10 mm) and large (> 10 mm). A large follicle of 10 mm or greater in diameter on the day before GnRH administration was considered morphologically to be a dominant follicle. Ovulation was determined by the disappearance of the dominant follicle. The day of the emergence of a new follicular wave was defined by the small follicles group above 4-5 mm. Desired images were frozen on the screen and measurements were taken.

Descriptive statistics was used and the data was presented as the mean ± standard error of mean (SEM). All tests were performed using graph pad prism version 8.0 for windows, graph pad software, San Diego California USA. WWW.graphpad.com. Values of p < 0.05 were considered statistically significant.

RESULT

Table 1. SUMMARY OF OVARIAN FOLLICULAR DYNAMICS AS DETERMINED BY UTRASONOGRAPHY IN BUNAJI CROSS-BRED COWS SUBJECTED TO OVSYNCH SYNCHRONISATION PROTOCOL

	FRIESIAN x BUNAJI	
	Group 1	Group 2
Occurrence of regression/ovulation after 1 st GnRH analogue injection (%)	0	60
New wave emergence after 1 st GnRH analogue injection (%)	0	60
Occurrence of ovulation after 2 nd GnRH analogue injection. (%)	20	80
Day by which ovulation had occurred post 2 nd GnRH injection (day)	1.40±0.61 ^a	2.0±0.55 ^b

Table 2. DIAMETER OF DOMINANT AND SUBORDINATE FOLLICLES IN BUNAJI CROSS-BRED COWS SUBJECTED TO OVSYNCH SYNCHRONIZATION

	FRIESIAN x BUNAJI	
	Group 1	Group 2
Size of dominant follicles at time of 1 st GnRH analogue injection (mm).	8.80±1.02 ^a	9.00±3.69 ^a



Size of pre-ovulatory follicle at 2 nd GnRH analogue injection (mm)	11.80±1.20	11.80±1.50
Rate of pre-ovulatory follicle growth after 2 nd GnRH analogue injection (mm).	1.43±0.06	0.99±0.55
Peak size of Pre-ovulatory follicle after 2 nd GnRH analogue injection (mm)	13.80±0.39	14.0±0.37
Diameter of largest subordinate follicle at ovulation (mm).	11.60±0.51 ^a	12.00±0.45 ^a

Group 1 All animals received 2 ml normal saline at the time of hormonal treatments

Group 2 GnRH: 100 µg + PGF₂α; 500 µg + GnRH 100 µg

RESULTS

1. OVARIAN FINDINGS

1.1 FOLLICULAR DYNAMICS

As shown in table 2, the sizes of dominant follicles at time of first GnRH analogue injection, sizes of pre-ovulatory follicles at second GnRH analogue injection, peak sizes of pre-ovulatory follicles after second GnRH injection and diameters of largest subordinate follicles of ovulation in the control and treated groups did not show any significant variation along the experiment period. Moreover, the characteristics of new follicular wave emergence differed significantly $P < 0.05$ in the two groups following the first GnRH administration.

1.2 OVULATORY RESPONSE

The cows in the control group had 0% and 20% ovulation rate at the time of first and second GnRH administrating respectively. While in the treated group ovulation occurred in 60% and 80% of cows as at the time of first and second GnRH administration respectively (Table 1). Ovulation occurred within 48 to 72 hours after the GnRH treatments and the emergence of a new follicular wave 1 to 4 days after the GnRH treatment (Mean ± SEM, 14 ± 060 days).

An accessory corpus luteum was detected in all treated animals between 2 - 4 days after the GnRH administration.

DISCUSSION

The present study indicates that ovulation rates obtained in the untreated group were 0% and 20% as at the time of first and second hormonal administration respectively. Lack of ovarian activity or time anoestrus is considered as one of the major problems to efficient cattle production and cows with true anoestrus may have complete ovarian inactivity (Islam *et al.*, 2013).

Rahman (2010) demonstrates that cows with true anoestrus by 60 days post-partum need to have some sort of management or hormonal interventions for induction of cyclicity. Accordingly, the current study has demonstrated that administration of GnRH analogue at the dosage indicated has positive effect on ovulation synchronization in Bunaji cross-bred cows (60 % and 80 % at the time of first and second hormonal administration respectively). In the present study interval after GnRH to ovulation was 30 - 36 hours, while 24 -32 hours have been reported in cattle (Pursely *et al.*, 1995; Wiltbank *et al.*, 2011) when a dominant follicle greater than 8.5 mm is present at the time treatment. Some authors (Martinez *et al.*, 1999; Perry and Perry 2009; Tortorella *et al.*, 2013) have reported a low ovulation rate despite large follicle diameters at the time of GnRH administration. This may imply that other factors apart from follicle size may influence ovulation rate. The present study also indicated the emergence of a new follicular wave 24 – 36 hours after ovulation. This is consistent with previous studies reporting emergence of new follicular wave within 3 – 4 days after GnRH treatment (Martinez *et al.*, 1999; Bo *et al.*, 2002). It has been reported that only if the first GnRH treatment results in ovulation do a synchronized follicular wave emergence occur (Bo *et al.*, 2002). However, GnRH did not induce ovulation in some cows that received PGF₂α. This may be due to lack of luteolysis after PGF₂α treatment thereby suppressing the development of the second follicular wave; or possibly that inadequate dose of GnRH or possibly that the follicle has lost its dominance (Carvalho *et al.*, 2015). The diameter of the dominant follicle of Bunaji



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crossbred cows (14.80 ± 0.37 mm) compared favourably to that obtained in *B. taurus* (14 – 18 mm; Sartori *et al.*, 2016) and *B. indicus* (14.1 – 15.7 mm; Uchechukwu, 2017).

CONCLUSION

From the current study, we concluded that Ovsynch protocol initiated at any stage of the oestrus cycle in Bunaji cross-bred cows could be beneficial in improving ovarian functions and reproductive potential of this breed. Ultrasonic imaging is very important and useful technique in the study of patterns of follicular developments.

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