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Research Article

# Follicular Wave Synchronization Using GnRH Agonist in Jersey Crossbred Cows

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## ABSTRACT

The study was aimed at recording the synchronization of follicular wave emergence in Jersey crossbred cows when administered with GnRH on Day 5 of the cycle. Initially, as a control study, the normal follicular wave pattern were studied in Jersey crossbred cows (n=6) using ultrasound scanner. During the subsequent cycle, all the animals were injected with GnRH agonist – Buserelin acetate (Inj. Receptal; 10 µg i.m.) on Day 5 of the cycle and follicular wave pattern was studied. Monitoring the normal follicular wave pattern revealed that, out of six cows, three cows (50%) exhibited two waves and three (50%) exhibited three waves per cycle with the mean days of emergence on 1.33 ± 0.90 and 10.0 ± 0.34 and 1.67 ± 0.34, 9.67 ± 0.67 and 15.33 ± 0.88 respectively. Administration of GnRH on day 5 of the cycle caused ovulation of dominant follicle (8.92 ± 0.60 mm) a new follicular wave emerged by two days of GnRH administration (Day7) in all the animals. In response to the GnRH administration normal two wave cycles were altered to three wave cycles with the third wave emerging on the mean day of 14.67± 1.20 and there was no significant change among the three wave cycles. There was no significant difference in the cycle length among the normal and GnRH treated cows (22.50 ± 0.83 vs 21.33 ± 1.49 days).

**Keywords:** Follicular wave synchronization – GnRH – Jersey crossbred cows

## INTRODUCTION

Follicular development could be synchronized by mechanical procedures or hormonal treatments. Gonadotropin-releasing hormone (GnRH) has been indicated as a tool for control of ovarian function in cattle when applied close to the time of insemination to induce ovulation and enhance conception rate (Morgan and Lean 1993). During recent years, emphasis has been put on the use of GnRH during the luteal phase, especially early or mid-dioestrus, as single or multiple injections to positively alter the follicular developmental patterns (Townson *et al.* 2002). Research efforts have been devoted to the development of treatment protocols that utilize GnRH during early or mid luteal phases in *Bos taurus* cows and heifers (Pursley *et al.* 1995). However, the incorporation of GnRH in follicular wave manipulation studies have not been reported in crossbred cows. So, the present study was aimed at recording the variations in follicular wave patterns of Jersey crossbred cows when administered with GnRH on early dioestrus stage (Day 5) of the cycle.

## MATERIALS AND METHODS

Six healthy and regularly cyclic Jersey crossbred multiparous cows (5 to 6 yrs) maintained at the Centralised Embryo Biotechnology Unit, Department of Animal Biotechnology, Madras Veterinary College, Chennai, were utilized for the study. All the cows were maintained under ideal and identical stall fed conditions through out the study. All the experimental cows were monitored regularly for oestrus symptoms and cyclicity of the animals was confirmed by frequent rectal examination.

Initially, as a control study, the normal follicular wave pattern in all the six cows were studied using a real time B-mode ultrasound scanner (SONOVET 600, SA-600V, KretzTechnik A.G, Austria), equipped

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with 7.5 MHz linear array transrectal transducer. The ovaries of each cow were examined every other day throughout an oestrous cycle starting from observed oestrus (Day 0) to subsequent standing oestrus (Sianangama and Rajamahendran, 1996). Diameter of corpus luteum (CL) and follicles were determined by taking the mean of the length and width of the structures (Zeitoun *et al.* 1996). A total of 12 normal cycles (two cycles per cow) were studied. During each examination, a sketch of the ovaries was made recording the location and diameter of CL and of individual identified follicles of $\geq$ 4 mm. If the follicle was not detected until it was $\geq$5 mm, a growth rate of 1.5 mm / 24 h was used to retrospectively determine the first examination when the follicle would have been $\leq$ 4.5 mm. The day of wave emergence was determined as the day the dominant follicle was first detected or retrospectively identified at a diameter of 4 -5mm (Bergefelt *et al.* 2003).

During the subsequent cycle, all the animals were injected with GnRH agonist- Buserelin acetate (Receptal® VET, Intervet International, GmbH, Germany; 10 µg i.m.) on Day 5 of the cycle and follicular wave pattern was studied as mentioned previously except, that ovaries were scanned daily from Day 4 to Day 9 to assess the fate of first wave dominant follicle and the emergence of subsequent wave. Ovulations resulting from treatment were verified by the disappearance of the dominant follicle present at the time of administering the GnRH. The emergence of an accessory corpus luteum (ACL) was characterized by luteal tissue appearing on a site previously occupied by the dominant follicle (Rajamahendran *et al.* 1998).

The data were statistically analysed as described by Snedcor and Cochran (1989).

## RESULTS AND DISCUSSION

Monitoring the normal follicular wave pattern revealed that, out of six cows, three (50%) cows exhibited two waves and the other three (50%) exhibited three waves per cycle. The mean days of emergence of follicular waves in two wave cycles were 1.33 $\pm$ 0.90 and 10.0 $\pm$ 0.34, while in three wave cycles the waves emerged on days 1.67 $\pm$ 0.34, 9.67 $\pm$ 0.67 and 15.33 $\pm$ 0.88. Zeitoun *et al.* (1996) stated that the greater proportion (>50%) of *Bos indicus* cows had three follicular waves per cycle, while Wolfenson *et al.* (2004) indicated that two wave cycles were more abundant (79%) than three wave cycles (21%) in *B. taurus* cows. In concurrence with this study similar days of emergence of follicular waves in two and three wave oestrous cycles were reported in *B. taurus* cows by Ginther *et al.* (1989) and Wolfenson *et al.* (2004) and in *B. indicus* cows by Zeitoun *et al.* (1996).

Irrespective of the wave pattern, it was evident that the dominant follicle of the first wave was in the growing phase on Day 5 with a mean diameter of 8.92 $\pm$ 0.60 mm. Administration of GnRH on Day 5 of the cycle caused ovulation of this dominant follicle and formation of ACL in all animals (100%). Usually the dominant follicle recruited from the first follicular wave occurring either during metoestrus or dioestrus will become atretic (Ireland *et al.* 2000). However, an injection of GnRH on day 5 induced ovulation or luteinization of that dominant follicle. Martinez *et al.* (2003) stated that approximately 3 days after the emergence of a follicular wave, dominant follicles (9 mm size) acquire LH receptors during the selection process of growth phase and Howard *et al.* (2006) reported that administration of GnRH on day 5 of the cycle caused secretion of both LH and FSH, overriding the negative feedback of progesterone on the anterior pituitary, resulting in ovulation or luteinization of the follicle, and subsequent formation of an additional CL. Our findings were in concurrence with these reports.

In response to GnRH administration, the first follicular wave was abruptly terminated by the ovulation of the dominant follicle and a new follicular wave emerged by two days (Day 7) after GnRH administration in all the animals (100%), as depicted in Fig.1 and 2. Removal of the suppressive effect of the dominant follicle by way of induced ovulations created a permissive environment, thus allowing for a new wave of follicles to emerge earlier (Rajamahendran *et al.* 1998). The mean interval from GnRH treatment to follicular wave emergence (2.0 days) in this study was in concurrence with Diskin *et al.* (2002) and Kim and Kim (2007). However, an interval of 2 - 4 (2.9) days for follicular wave emergence was reported by Kim *et al.* (2005). Macmillan *et al.* (2003) stated that manipulation of follicular development by synchronizing new wave emergence has been most successful using GnRH only when injected after follicle deviation and the establishment of dominance, which would induce their ovulation. Similarly, a study by

FIG 1. EFFECT OF GnRH ON TWO FOLLICULAR WAVE CYCLES

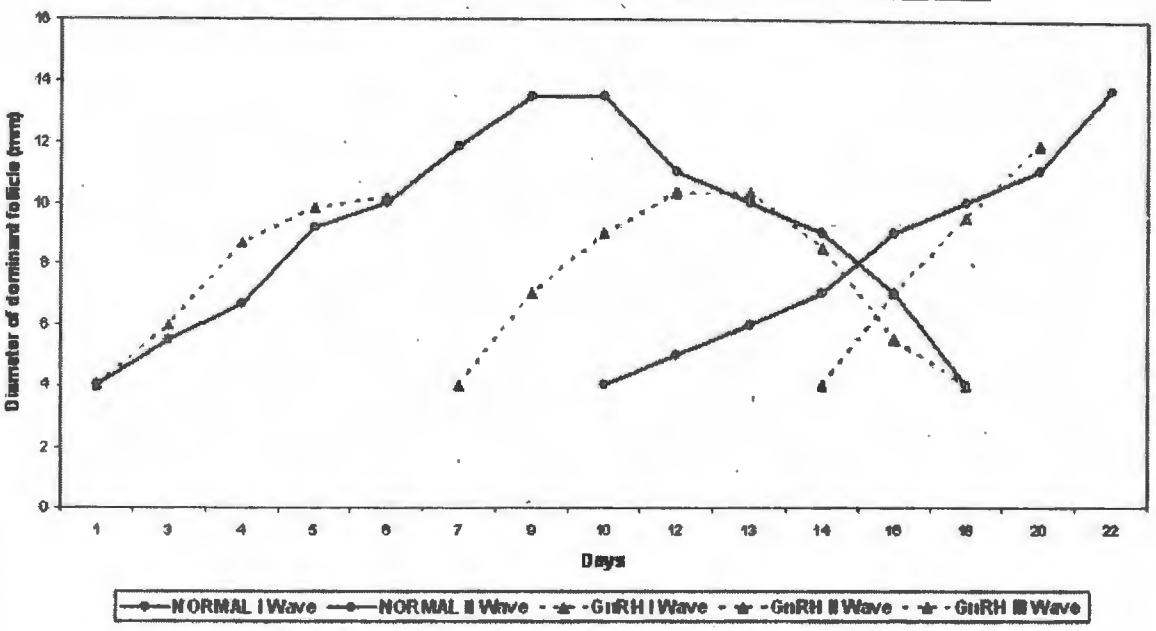
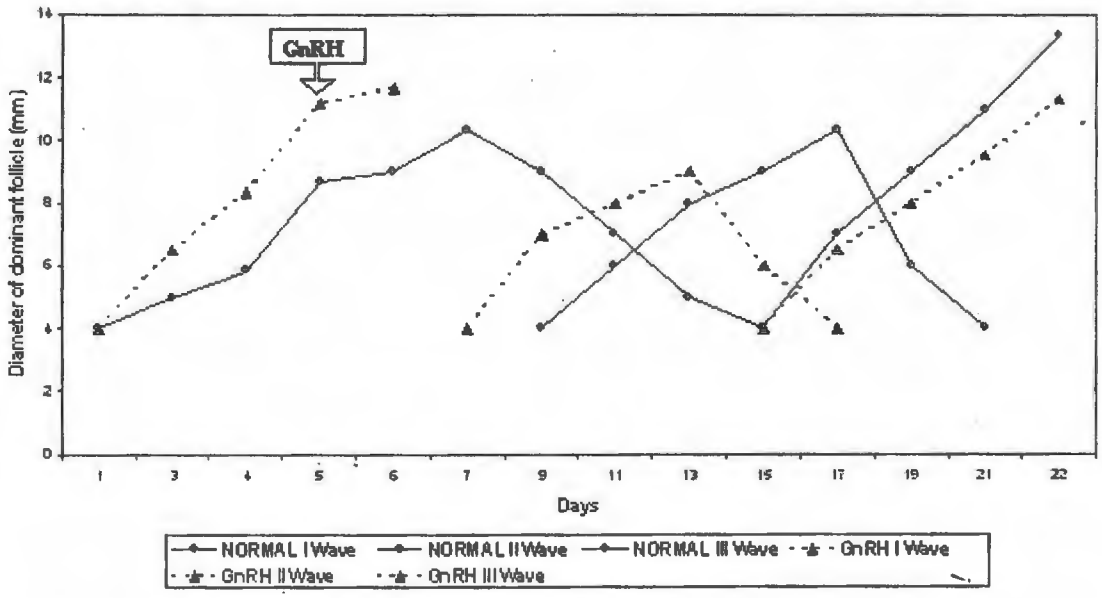



FIG 2. EFFECT OF GnRH ON THREE FOLLICULAR WAVE CYCLES




Bo *et al.* (2002) confirmed that GnRH does not always result in ovulation or luteinization of the dominant follicle and the emergence of a new follicular wave was synchronized only when the treatment caused ovulation. Ryan *et al.* (1998) found that the development of the follicle wave continued unaltered if the GnRH injection occurred before follicle dominance was established.

Interestingly, in all cows that exhibited two wave cycles normally, GnRH treatment altered them to three wave cycles with the third wave emerging on the mean day of 14.67± 1.20 as against on day 15.33 ± 0.88 in control cycles. The second (synchronized) follicular wave emerged earlier in GnRH treated (Day 7.0) than control animals (Day

9.67). Furthermore, there was a significant decrease in the maximum diameter ($P<0.05$) and a non-significant decrease in growth phase of the second (induced) follicular wave dominant follicle in GnRH treated than control cycles. This dominant follicle also started regressing much earlier than in normal cycles, which might be due to the suppressive effect of high progesterone secreted by both CL and ACL as suggested by Viana *et al.* (2000). Thus early emergence, short period of dominance and faster regression of second follicular wave dominant follicle would have resulted in the emergence of third follicular wave.

The mean length of oestrous cycle for the crossbred cows under study was 22.50 $\pm$ 0.83 days. The cycle length was non-significantly longer in three wave cycles (22.67 days) when compared with two wave cycles (22.33 days). There was no significant difference in the cycle length among the normal and GnRH treated cows (22.50 $\pm$ 0.83 vs 21.33 $\pm$ 1.49 days). The present finding was in accordance with Macmillan *et al.* (2003) who also reported that there was no effect on cycle length if the GnRH injection was in the first half of the cycle.

Thus, GnRH administration on Day 5 of the oestrous cycle in crossbred cows caused i) Ovulation of the first wave dominant follicle with formation of ACL ii) Synchronized emergence of follicular wave by two days after GnRH administration and iii) Two wave cycles were altered to three wave cycles. Sato *et al.* (2005) opined that GnRH was effective to synchronize follicular wave emergence as a pretreatment for superstimulation. Willard *et al.* (2003) stated that the increase in CL tissue in cows treated with GnRH after insemination patterned the increased serum concentrations of progesterone and facilitated a protective effect within endocrine environment of the uterus to avoid early embryonic mortality around the time of maternal recognition of pregnancy. Townson *et al.* (2002) also reported that cows which had a three-wave cycle preceding insemination did have higher conception rates to first insemination than those with two waves which could be because the ovulatory follicle developed over a shorter period. Thus it was concluded that follicular wave emergence could be positively synchronized by administration of GnRH on Day 5 of the oestrous cycle in crossbred cattle.

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