

# Effect of increasing GnRH dose during Double-Ovsynch on fertility of lactating dairy cows

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## Introduction

The rate of ovulation to the first GnRH treatment of Ovsynch-type protocols is key to achieve excellent fertility. In cows synchronized with the Double-Ovsynch protocol (DO), only around half of the cows ovulate to the third GnRH injection (first GnRH of the Breeding-Ovsynch portion of Double-Ovsynch). Increasing the dose of GnRH at this time has been proven to improve ovulatory response, reaching up to 67% of ovulation rate. Our objective was to determine whether increasing the dose of the third GnRH of DO could improve the reproductive outcomes at first service.

## Materials and methods

The study was conducted in Torreon, Mexico from December 2021 to March 2022. Lactating Holstein cows ( $n = 1096$ ; primiparous = 401, multiparous = 695) received first service timed AI (TAI) with conventional semen at  $74 \pm 3$  DIM (primiparous) or  $67 \pm 3$  DIM (multiparous) after synchronization with DO [GnRH-7 d-PGF-3 d-GnRH-7 d-GnRH-7 d-PGF-24 h-PGF-32 h-GnRH-16 to 20 h-TAI]. Cows were enrolled in DO weekly at  $47 \pm 3$  DIM (primiparous) or  $40 \pm 3$  DIM (multiparous) and randomly allocated to receive a regular dose of GnRH (CTRL; 10.5 mcg of buserelin acetate; 2.5 mL of Gonaxal®, Biogenesis Bago) or an increased dose of GnRH (GnRH2x; 21 mcg of buserelin acetate; 5 mL of Gonaxal, Biogenesis Bago) at the third GnRH injection of DO (first GnRH of the Breeding-Ovsynch portion of DO; i.e.,  $64 \pm 3$  DIM for primiparous and  $57 \pm 3$  DIM for multiparous cows). All other injections of DO were set at 10.5 mcg of buserelin acetate (2.5 mL Gonaxal, Biogenesis Bago) for GnRH and at 150 mcg of D-Cloprostenol (2 mL of Croniben®, Biogenesis Bago) for PGF. Pregnancy diagnosis and reconfirmation of pregnancy were conducted by transrectal ultrasound at  $32 \pm 3$  and  $63 \pm 3$  days since insemination, respectively. Binary outcomes (i.e., pregnancies per artificial insemination [P/AI], pregnancy losses, and final proportion of cows pregnant) were analyzed by logistic regression using the PROC GLIMMIX (SAS 9.4, SAS Institute Inc.). The effect of treatment (CTRL vs GnRH2x), parity (primiparous vs multiparous), and treatment by parity interaction were offered as fixed effect in all the models. All explanatory variables were considered significant if  $P \leq 0.05$ , while  $0.05 < P \leq 0.10$  was considered a tendency. Results are reported as LSM  $\pm$  SEM.

## Results

From the cows enrolled, 4% ( $n = 41$ ; CTRL = 16, GnRH2x = 25) were classified as do not breed or left the herd before receiving TAI, while 3% ( $n = 29$ ; CTRL = 13, GnRH2x = 16) were inseminated but left the herd before knowing their pregnancy status. Thus, 92% ( $n = 935$ ; CTRL = 472, GnRH2x = 463) were included in the analysis. P/AI were similar between groups ( $P = 0.720$ ; CTRL =  $47.3 \pm 2.4\%$ , GnRH2x =  $48.6 \pm 2.4\%$ ), but greater for primiparous than multiparous cows ( $P < 0.001$ ; primiparous =  $55.2 \pm 2.7\%$ , multiparous =  $40.8 \pm 2.0\%$ ). No treatment by parity interaction was observed for P/AI ( $P = 0.769$ ; primiparous: CTRL =  $54.1 \pm 3.8\%$ , GnRH2x =  $56.3 \pm 3.8\%$ ; multiparous: CTRL =  $40.7 \pm 2.8\%$ , GnRH2x =  $40.9 \pm 2.9\%$ ). Pregnancy losses were similar between groups ( $P = 0.515$ ; CTRL =  $6.6 \pm 1.8\%$ , GnRH2x =  $5.1 \pm 1.5\%$ ), parity ( $P = 0.653$ ; primiparous =  $5.3 \pm 1.6\%$ , multiparous =  $6.4 \pm 1.6\%$ ) and treatment by parity interaction ( $P = 0.532$ ; primiparous: CTRL =  $5.4 \pm 2.3\%$ , GnRH2x =  $5.3 \pm 2.3\%$ ; multiparous: CTRL =  $8.2 \pm 2.5\%$ , GnRH2x =  $5.0 \pm 2.0\%$ ). The final proportion of cows pregnant was similar between groups ( $P = 0.587$ ; CTRL =  $44.1 \pm 2.4\%$ , GnRH2x =  $46.0 \pm 2.4\%$ ), but greater for primiparous than multiparous cows ( $P < 0.001$ ; primiparous =  $52.2 \pm 2.7\%$ , multiparous =  $38.1 \pm 2.0\%$ ). No treatment by parity interaction was observed for total proportion of cows pregnant ( $P = 0.939$ ; primiparous: CTRL =  $51.2 \pm 3.8\%$ , GnRH2x =  $53.3 \pm 3.9\%$ ; multiparous: CTRL =  $37.3 \pm 2.8\%$ , GnRH2x =  $38.9 \pm 2.8\%$ ).

## Significance

Increasing the dose of GnRH at the third GnRH injection of DO in lactating dairy cows did not affect fertility or pregnancy losses at first service.

