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# Lengthening the superstimulatory treatment protocol increases ovarian response and number of transferable embryos in beef cows

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**Abstract**

This study determined if lengthening the superstimulation protocol from 4 to 7 days would result in an increase in the superovulatory response with no adverse effects on oocyte/embryo competence in beef cows. Follicular ablation was performed, a progesterone-releasing intravaginal device (PRID) was inserted, and cows were assigned to one of two treatment groups 5 to 8 days after ovulation: Control (4 days of follicle stimulating hormone (FSH)) or Long (7 days of FSH; n = 12 per group). The FSH treatments were initiated 1.5 days later (Day 0). A dose of 400 mg NIH-FSH-P1 (Folltropin-V) was distributed equally over 8 (Control) or 14 (Long) im injections at 12-h intervals. Prostaglandin F2$\alpha$ (PGF) was administered twice, 12 h apart, on Day 2 (Control) or Day 5 (Long), and PRID were removed 12 h after the second PGF. Both groups were given 25 mg pLH (lutropin-V) im 24 h after PRID removal and AI was done 12 and 24 h later. Ova/embryos were collected 7 days after the pLH injection. The mean ($\pm$ SEM) number of $\geq$ 9 mm follicles at the time of first AI did not differ (P = 0.24) between groups, but more ovulations (30.9 $\pm$ 3.9 vs. 18.3 $\pm$ 2.9, P = 0.01) and CL (27.2 $\pm$ 2.1 vs. 20.8 $\pm$ 2.2, P = 0.04) occurred in the Long group. A higher proportion of the $\geq$ 9 mm follicles ovulated between 12 and 36 h after pLH in the Long group (93 vs. 69%; P = 0.001). Although numerically higher in the Long group, mean numbers of total ova/embryos, fertilized ova, transferable or freezable embryos did not differ. In conclusion, a lengthened superstimulatory treatment protocol resulted in more follicles acquiring the capacity to ovulate with an increased number of ovulations, and without a decrease in oocyte/embryo competence.


*Keywords:* Cattle; Superstimulation protocol; Follicular development; FSH; Progesterone

## 1. Introduction

Multiple ovulation and embryo transfer (MOET) programs have been used worldwide to increase the number of offspring from highly valued bovine donors. The objective of superstimulatory treatments is to increase the number of ovulations of competent follicles to maximize numbers of transferable embryos [1]. Despite advances in the understanding of ovarian follicular dynamics, variability of response in donor females remains one of the major limiting factors affecting the success of MOET programs in cattle [2,3].

Most estrous cycles in cattle consist of either two or three follicular waves [1,4,5]. A follicular wave is defined as the synchronous growth of a group of small follicles (from 4 to 5 mm in diameter) from which a

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single follicle is selected to become the dominant follicle, whereas the others (subordinate follicles) undergo regression [1,5]. Each follicular wave is preceded by a surge in circulating follicle stimulating hormone (FSH) concentrations [6,7], and intrafollicular factors, produced mainly by the dominant follicle, have a suppressing effect on circulating FSH concentrations which is crucial for the selection process [4–6,8], and to ensure monovulation by regression of subordinate follicles [9]. Superstimulatory treatments with exogenous FSH prevent regression of subordinate follicles; the response is strongly influenced by follicular wave status at the start of treatment [10,11].

Follicles as small as 1 mm in diameter are capable of initiating growth under the influence of FSH [12]. It has been suggested that the addition of 2 to 3 days to a conventional superstimulation protocol will allow small follicles to be recruited into the cohort of growing follicles [13]. In this regard, Caccia, et al. [14] reported an increase in the superovulatory response in cattle when a small dose of equine chorionic gonadotropin (eCG) was administered 2 days before the beginning of FSH treatments. Furthermore, a 6-days superstimulation protocol starting at random stages of the estrous cycle and without prior synchronization of follicle wave emergence resulted in a comparable number of ovulations and ova/embryos as a 4-day treatment protocol initiated at follicle wave emergence [13]. Finally, in a recent study, beef cows were superstimulated with FSH in a 4- or 7-days protocol to mimic the length of the growing phases of 3- vs. 2-wave dominant follicles. The 7-day treatment protocol resulted in a prolonged growing phase and a higher number of ovulatory-sized follicles; however, this may have been because of more FSH being administered over the longer treatment protocol [15].

The present study was designed to test the hypothesis that an increase in the length of the superstimulatory treatment protocol from 4 to 7 days with the same total dosage of FSH will result in an increase in the numbers of ovulatory-sized follicles, subsequent ovulations and viable embryos with no adverse effects on oocyte/embryo competence.

## 2. Materials and methods

### 2.1. Animals

The experiment was conducted at the Goodale Research Farm (52° N and 106° W), University of Saskatchewan, during the experimental period of November to December 2010. Twenty-four cross-breed beef cows were housed outdoors in a feedlot pen and fed mixed grass hay, with free access to salt, mineral mix and water. Animal procedures were performed in accordance with the Canadian Council on Animal Care and were approved by the University of Saskatchewan Protocol Review Committee.

### 2.2. Pretreatment procedures and animal grouping

At random stages of the estrous cycle, cows were subjected to ultrasound-guided transvaginal ablation of all follicles ≥5 mm to synchronize the emergence of a new follicular wave, expected ≈1.5 days later [7]. Transrectal ultrasonography was performed with a B-mode ultrasound scanner equipped with a 7.5 MHz linear-array transducer (MyLabFive VET, Biosound Esaote, IN, USA) and follicles ≤5 mm at the time of wave emergence were counted to determine the numbers of follicles in the wave. A balanced randomization of animals was then conducted to insure that cows with comparable numbers of follicles were equally represented within each treatment group [16,17].

The two treatment groups were referred to as Control (4-day protocol) and Long (7-day protocol) groups. Cows assigned to the Long group were given a single dose of prostaglandin F2$\alpha$ (PGF, 500 $\mu$g of D-cloprostenol; Estroplan, Vétoquinol, Lavaltrie, QC, Canada) intramuscularly (im) 7 days after follicular ablation. Two days later, the same treatment was applied to the Control group. This strategy was employed to insure that cows from both treatment groups would be represented on each day of embryo collection (a total of five collection days). Starting 2 days after PGF injection, transrectal ultrasonography was performed every 24 h to detect ovulation, defined as the disappearance of the largest follicle between two consecutive observations [18]. Transvaginal ultrasound-guided follicular ablation of all follicles ≥5 mm was then performed on all cows in the afternoon 5 to 8 days after ovulation and the expected day of wave emergence (1.5 days after ablation) was defined as Day 0 [7] (Fig. 1).

### 2.3. Superstimulation protocols

A progesterone releasing intravaginal device (PRID, Vétoquinol) was inserted immediately after follicle ablation (Day −1.5) and superstimulatory treatments were initiated the morning of Day 0 (Fig. 1). The Control group was given 400 mg NIH-FSH-P1 (Folltropin-V; Bioniche Animal Health, Belleville, ON, Canada) distributed over eight im injections at 12 h