# Superovulation of lactating Holstein cows with a single dose of recombinant bovine FSH in a commercial dairy setting in Brazil

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Objective:

Until recent times, gonadotropin FSH for superovulation in cattle was primarily extracted from the pituitary of slaughtered animals, mainly pigs. However, this method presented several drawbacks, including potential contamination, pathogen transmission and batch-to-batch inconsistency. Recently, alternative production methods utilizing recombinant technology have been licensed to produce a bovine recombinant FSH homologue. Therefore, the aim of this study was to evaluate the effectiveness of a single injection long-acting recombinant bovine FSH on superovulation for *in vivo* embryo production in lactating Holstein primiparous cows.

Materials and Methods:

Twenty primiparous lactating Holstein cows [days in milk (DMI)= 99.8 ± 6.80 and milk production (MILK) per day = 53.8 ± 1.53 kg], sourced from Agrindus farm (Descalvado, SP, Brazil) at a random stage of their estrous cycle were subjected to a synchronization program. On D0, cows received 2 mg of estradiol benzoate and the introduction of a progesterone-releasing intravaginal device (1,55g of progesterone). Four days later, the cows received 6ml of recombinant bovine FSH (Zimbria®, Ceva Sante Animale, France); equivalent to 300 mcg of rb-FSH. On day 6 a.m, the first injection of PGF 2alpha (150 mcg of D-cloprostenol) was administered, followed by a second injection of PGF 2alpha on day 6 p.m. The progesterone device was removed on day 7 p.m., and on day 8 p.m. all the cows received GnRH (20 mcg of buserelin acetate). Donors received two artificial inseminations, one on day 9 a.m. and the second one on day 9 p.m. All the cows were flushed on day 15 p.m. The number of viable embryos, degenerated embryos and unfertilized oocytes were collected and correlated with the number of corpora lutea (CL) present on both ovaries at flushing time, evaluated by transrectal ultrasonography. Cows were classified as high DIM (121.7 ± 8.32 days in milk, n= 10) and low DIM (77.9 ± 4.44 days in milk, n=10). Also, cows were classified as high MILK (59.5 ± 1.14 Kg/day, n= 10) and low MILK (48.1 ± 1.18 Kg/day) to evaluate the number of CL, number of viable embryos, recovery rate, unfertilized embryo rate and degenerate embryo rate. Statistical analyses were performed by SAS Glimmix procedure (v9.4).

Results:

No interaction was found between DIM and MILK for any variables ($P > 0.05$). The number of CL did not differ between DIM groups (High DIM= 12.7 ± 1.9 vs. Low DIM=

13.8 ± 3.3; P= 0.73) nor between MILK groups (High MILK= 12.3 ± 2.7 vs. Low MILK= 14.2 ± 2.7; P= 0.47). No differences were observed for number of viable embryos between High and Low DIM (5.90 ± 1.4 vs. 4.10 ± 1.1; P= 0.35) nor between High and Low MILK (5.70 ± 1.40 vs. 4.30 ± 1.08; P= 0.54). The recovery rate was higher in High than Low DIM (76% vs. 63%; P= 0.03). However, no difference was found for MILK (High MILK= 73% vs. Low MILK= 67%; P= 0.32). The unfertilized embryo rate was similar between High or low DIM groups (27% vs. 21%; P= 0.97) and between High or low MILK groups (24% vs. 25%; P= 0.25). Furthermore, the degenerated embryo rate did not differ between High and Low DIM (13% vs. 19%; P=0.37) nor between High and Low MILK (8% vs. 23%; P= 0.16).

Conclusions:

The study investigated the efficacy of a single injection of long-acting recombinant bovine FSH for superovulation in lactating Holstein primiparous cows. The study showed the efficacy of the product in inducing multiple ovulations and multi embryonic developments. Results indicated no significant differences in the number of viable embryos, unfertilized embryos, or degenerated embryos between high and low DIM or MILK groups. However, a higher recovery rate was observed in cows with high DIM. These findings suggest that the recombinant bovine FSH homologue (Zimbria®, Ceva) offers a viable alternative to traditional pituitary extracts which required multiple injections for superovulation. Furthermore, the recombinant molecule can bring benefits in terms of safety and consistency. Further research and larger-scale studies are warranted to validate and expand upon these preliminary findings.

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