

## Nerve growth factor $\beta$ increases small luteal cell number and enhances steroidogenic activity in bovine corpus luteum

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Nerve growth factor  $\beta$  (NGF) is a seminal plasma protein that stimulates bovine theca cell proliferation and steroidogenesis in vitro and is luteotrophic in vivo, yet there is limited information on how it alters complex dynamics associated with ovulation and corpus luteum (CL) formation in cattle. Objectives were to evaluate the effects of systemically administered purified bovine NGF on vascularity and steroidogenic function of pre ovulatory follicles and subsequent CL in cattle. Our hypothesis was that systemic administration of NGF to heifers with a pre-ovulatory follicle increases vascularity and steroidogenic function of follicle, decreases the time to ovulation, and improves subsequent luteotrophic function through increased vascularity and small luteal cell number in the CL. Holstein heifers ( $n = 12$ ) were synchronized using a GnRH prostaglandin and intravaginal progesterone protocol (5 day CIDR-Synch) and randomly assigned to 1 of 2 treatment groups: 12 ml phosphate-buffered saline (CONT) or 250  $\mu\text{g}$  purified NGF in 12 ml phosphate-buffered saline (NGF) given IM at time 0 (presence of pre-ovulatory follicle). Using a crossover design, a second replicate was performed at  $\sim 1$  month after completion of first replicate. Transrectal ultrasonography with Doppler and blood sampling were performed every 4 hours from 0 - 32 hours to evaluate follicle size and vascularity, time to ovulation, and serum estradiol concentrations. Transrectal ultrasonography was performed daily to assess CL size and vascularity, and blood was obtained every 2 days to measure serum progesterone concentrations. On days 9 and 14, CL biopsy was performed on a subset of heifers ( $n = 6$  heifers/treatment/time) to assess mRNA expression of steroidogenic enzymes and LH receptor by qPCR and to determine the histological ratio of small to large luteal cells. Statistical analyses were performed using a General Linear Mixed Model with repeated measures, using heifer ID as a random variable (R Version 3.4.3). There was a main effect of NGF treatment on follicle diameter ( $p = 0.02$ ), but no effect on serum estradiol concentrations ( $p = 0.95$ ) or time to ovulation ( $p = 0.42$ ). Treatment with NGF tended to increase CL diameter ( $p = 0.10$ ) and increased serum progesterone concentrations ( $p = 0.04$ ) from days 10 - 12. There was a higher ( $p < 0.01$ ) percentage of small luteal cells and a tendency for increased LH receptor gene expression ( $p = 0.09$ ) in the CL of NGF treated heifers. Consistently, there were increased expressions ( $p \leq 0.05$ ) of genes for steroidogenic acute regulatory protein and  $3\beta$ hydroxysteroid dehydrogenase in the CL of NGF treated heifers. Treatment with NGF did not alter vascularity ( $p \geq 0.16$ ) of the follicle or CL. We concluded that systemic administration of purified NGF to heifers interacted with pre ovulatory follicles to alter downstream CL formation and function. Luteotrophic effect of NGF was attributed to increased small luteal cell number and enhanced steroidogenic enzyme activity within the CL.

**Keywords:** Bovine, luteotrophic, nerve growth factor  $\beta$ , ovulation, progesterone

### Acknowledgement

This project was funded by the United States Department of Agriculture - National Institute of Food and Agriculture (Hatch Funds # 1014712).

