

Direct effects of nerve growth factor β , purified from bull seminal plasma, on steroidogenesis and angiogenic markers of the bovine preovulatory follicle

Jamie Stewart,^{a,b} Liying Gao,^a Jodi Flaws,^a Vitor Mercadante,^c Nicholas Dias,^c

Igor Canisso,^a Fabio Lima^a

^aCollege of Veterinary Medicine, University of Illinois, Urbana, IL

^bVirginia-Maryland College of Veterinary Medicine
Virginia Polytechnic Institute and State University, Blacksburg, VA

^cCollege of Agricultural and Life Sciences
Virginia Polytechnic Institute and State University Blacksburg, VA

Nerve Growth Factor β (NGF) is a seminal plasma protein that induces ovulation and has a luteotrophic effect in camelids. In spontaneously ovulating species, NGF signaling in the ovary is critical for the first ovulation, but little is known regarding interactions of seminal plasma derived NGF on the preovulatory follicle. Objectives were to assess direct effects of purified bovine NGF on steroidogenesis and angiogenic markers in the bovine preovulatory follicle. Our hypothesis was that NGF administration stimulates steroidogenesis and angiogenic markers in thecal and granulosa cells from the bovine preovulatory follicle. Two Holstein heifers were synchronized using a 5 day CIDR Synch (day 0; GnRH + CIDR insert, day 5; PGF_{2 α} + CIDR removal, day 6; PGF_{2 α}). Ovariectomy was performed via colpotomy 48 hours after the second PGF_{2 α} injection when a pre-ovulatory follicle > 12 mm was present. Preovulatory follicle was excised from the ovary, fluid was aspirated and tissue dissected into quarters. Theca interna with adherent granulosa cells was peeled from the theca externa and surrounding stromal tissue and cut into small pieces (average weight: 5.3 $\pm$ 0.7 mg). Follicle tissue pieces were incubated (37°C, 5% CO₂:95% air) in 0.5 ml of Eagle's MEM culture media supplemented with 1% L glutamine, 1% nonessential amino acids, 1% penicillin streptomycin, 1% insulin-transferrin-selenium, 10% fetal bovine serum, 40 ng/ml cortisol, 4 ng/ml LH, and 4 ng/ml FSH. Culture wells were either supplemented with 100 ng/ml NGF (n = 12) or left as an untreated control (n = 12). Media was withdrawn and replaced with fresh media at 3, 6, 12, 24, 48, and 72 hours of culture and frozen at -80°C pending hormone analyses. After 72 hours, follicle tissue pieces were flash frozen to measure steroidogenic and angiogenic gene expression using qPCR. Analysis of variance was applied to parametric data using a general linear mixed model, with repeated measures used for hormone data. A Kruskal Wallis rank sum test was performed on non-parametric data. Treatment with NGF increased (p < 0.01) testosterone production and upregulated (p = 0.04) steroidogenic enzyme 17 beta-hydroxysteroid dehydrogenase gene expression at 72 hours in follicle-wall extracts. There was no effect of NGF treatment on production (p $\geq$ 0.14) of progesterone or estradiol or gene expression (p $\geq$ 0.31) of other steroidogenic enzymes in the follicle. Additionally, treatment with NGF downregulated (p = 0.02) gene expression of the angiogenic fibroblast growth factor 2, but did not alter expression (p $\geq$ 0.44) of other angiogenic factors. Increased androgen production with NGF treatment may be secondary to theca cell proliferation. Based on reduced expression of fibroblast growth factor 2 in NGF treated cells, perhaps there was an early onset of cell remodeling during early corpus luteum development.

Keywords: Angiogenesis, cattle, follicle, nerve growth factor β , steroidogenesis

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