Developmental and hormonal regulation of gene expression of fibrillin-1 (FBN1) and the asprosin receptor, olfactory receptor family 4 subfamily M member 1 (OR4M1), in bovine ovarian cells

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BACKGROUND

- Asprosin is a novel protein encoded by *FBN1* gene and formed when FBN1 is cleaved at C-terminal end by enzyme furin (Duerrschmid et al., 2017).
- Asprosin is correlated with insulin resistance, type II diabetes and PCOS and functions via the G-protein-cAMP-PKA pathway and increase hepatic glucose level (Romere et al., 2016).
- Asprosin receptor, olfactory receptor (Olfr734/OR4M1) has been identified in liver, kidney, and testes (Li et al., 2019).

HYPOTHESIS: *FBN1* mRNA is expressed in ovarian follicular cells and is hormonally regulated.

OBJECTIVES

- To characterize *FBN1, furin* and *OR4M1* mRNA abundance in theca (TC) and granulosa cells (GC) during follicular development and the possible hormone regulators for the expression of *FBN1* mRNA.
- To identify the effects of asprosin on steroid production and proliferation of TC.

MATERIALS AND METHODS

- **Biological Materials.** GC and TC of small (<5mm) and large (>6 mm) follicles were obtained at abattoir. Cells were collected for RNA isolation or cultured with 10% fetal calf serum and after 48 h, treatments were applied in a serum-free medium for 24 h.
- **RNA extraction** was done using Trizol method.
- **Gene expression analysis** was made using one-step rt-PCR with 18s as reference gene. Data are presented as $2^{-\Delta\Delta Ct}$.
- **Cell counting and radioimmunoassay (RIA).** Cells were counted using Coulter Counter. Medium from culture plates were collected for double antibody RIA for quantification of progesterone and androstenedione production.
- **Statistical analysis.** Data were analyzed by 2x2 factorial (Exp. 1), 3x2 factorial (Exp. 2), one way ANOVA (Exp. 3) and 2x2 factorial (Exp. 4).

RESULTS

- **Experiment 1:** *FBN1, OR4M1* (Figure 1) and *furin* (data not shown) mRNA abundance varied with follicle development and cell type.
- **Experiment 2:** *FBN1* mRNA abundance in TC was increased ($P < 0.05$) by TGFβ1 (Figure 2), FGF2, FGF9 and EGF (data not shown).
- **Experiment 3:** TGFβ1, WNT3A and FGF9 increased ($P < 0.05$) *FBN1* mRNA abundance in GC and IGF1 decreased ($P < 0.05$) *FBN1* mRNA in GC in vitro (data not shown).
- **Experiment 4:** Asprosin inhibited (by 10%; $P < 0.05$) IGF-1 induced TC numbers, but had no effect on progesterone production ($P > 0.10$). Asprosin increased androstenedione production by 26% in LH treated TC ($P < 0.05$) (data not shown).

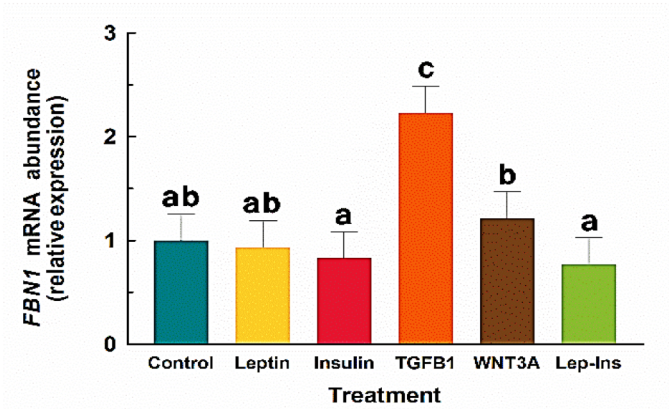
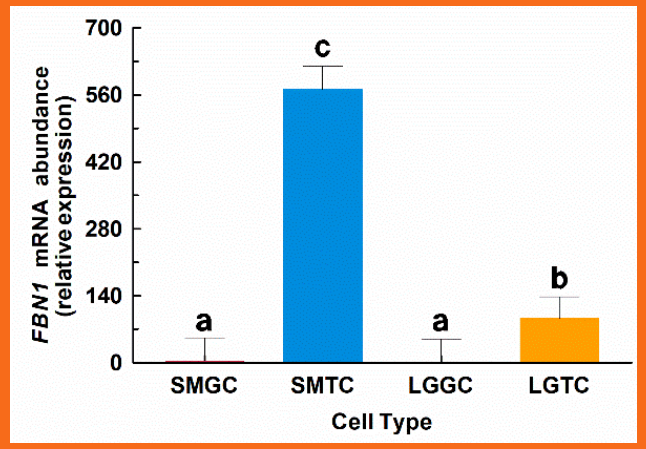


Figure 2: (Exp. 2) TGFβ1 induced *FBN1* mRNA abundance in theca cells. Bars without common letter differ ($P < 0.05$).

CONCLUSION: *FBN1* and *OR4M1* mRNA are developmentally and hormonally regulated in ovarian GC and TC and may perform a paracrine/ autocrine regulation of ovarian function in cattle by regulating cell proliferation and stimulating hormone-induced androgen production.

Literature Cited: *Duerrschmid et al., 2017, Nature Med. 23:1444. Li et al., 2019, Cell Metab. 30:319. Romere et al., 2016, Cell 165:566.*



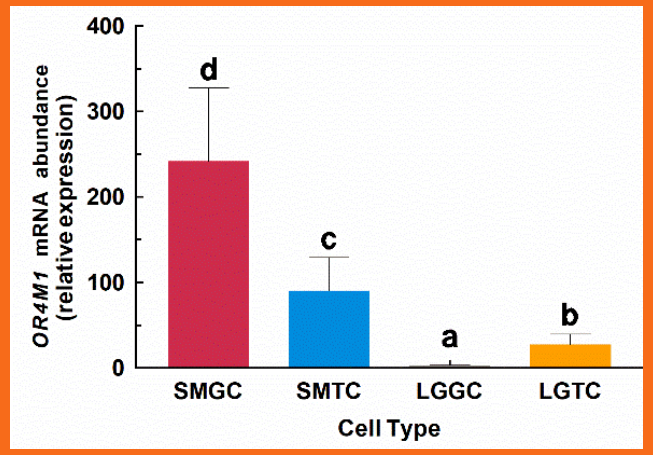


Figure 1 (Exp. 1). *FBN1* mRNA abundance is greater in TC than GC (top)**,** whereas, ***OR4M1* mRNA is greatest in small follicle GC** (bottom). SM-small, LG-large, GC-granulosa cells, TC-theca cells. Bars without a common letter differ ($P < 0.06$).

Asprosin may be acting as a autocrine/ paracrine regulator of ovarian follicular function in cattle.

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