Preovulatory Follicles of GnRH-II Receptor Knockdown Gilts Possess Fewer Hypertrophic Theca Cells, but Similar Numbers of Hypotrophic Granulosa Cells Compared with Littermate Controls

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The second form of gonadotropin-releasing hormone (GnRH-II) and its associated receptor (GnRHR-II) are only produced in one mammalian livestock species, the pig. Unlike the classical GnRH system, this ligand-receptor complex is more abundant in peripheral tissues. Regarding female reproduction, GnRH-II and its receptor are present in multiple ovarian-derived cell lines (human, primate, rodent) and GnRH-II has been discovered in human granulosa (GC) and theca (TC) cells. Recently, our laboratory measured GnRH-II within follicular fluid and detected GnRHR-II protein within both GC and TC of porcine preovulatory follicles. To examine how GnRH-II and its receptor mediate biological processes within preovulatory follicles, we utilized a transgenic swine line with ubiquitous knockdown (KD) of GnRHR-II (GnRHR-II KD). Preliminary studies revealed that GnRHR-II KD females: 1) secrete 20% less serum 17β-estradiol than littermate controls during peak concentrations [-44 to -8 h prior to initiation of behavioral estrus (0 h)] within the follicular phase of the estrous cycle, despite similar circulating levels of gonadotropins; 2) produce 40% less GnRHR-II protein in TC of preovulatory follicles compared with control TC or GC from either line; 3) ovulate 16% fewer follicles than control littermates, although they possess similar numbers of large antral follicles; and 4) possess preovulatory antral follicles that are 10% larger in diameter than corresponding follicles from control females. Therefore, the objective of the current study was to characterize the morphology of GC and TC within preovulatory follicles collected from GnRHR-II KD (n = 4) and littermate control (n = 4) gilts. Mature females were euthanized during proestrus (Day 18-20 of the estrous cycle). Follicle diameter was measured using vernier calipers and large antral follicles (≥ 6.5 mm) were excised from ovarian stroma, fixed in 4% paraformaldehyde, and paraffin embedded. Sequential sections (approximately 6-7 μm thick) were collected, mounted, and stained with hematoxylin and eosin. Sections with the largest measured follicular area were imaged (100X) at 8 random locations within the theca interna and mural GC layers. Cell numbers were quantified within a pre-set rectangular area; cell areas were determined using the closed polygon tool of the cellSens imaging software (Olympus Life Science, Waltham, MA) by tracing the plasma membrane of 40 GC and 40 TC exhibiting a defined plasma membrane and visible nuclei. Statistical analyses performed using the MIXED procedure of SAS included models for both cell area and cell number, with line as a fixed effect, litter as a random effect, and follicular area as a covariate. Log transformations were performed on all variables to meet normality assumptions. The number of GC within preovulatory follicles did not differ ($P > 0.05$) between lines; however, GC in GnRHR-II KD tended ($P < 0.06$) to be smaller (31.6 ± 0.9 μm^2) than those within littermate control (33.1 ± 0.9 μm^2) follicles. Interestingly, GnRHR-II KD females tended ($P < 0.08$) to possess fewer TC (38.4 ± 1.8 cells) than littermate controls (42.2 ± 1.8 cells). Moreover, TC from GnRHR-II KD follicles

were significantly ($P < 0.001$) larger (94.0 ± 3.2 μm^2) than TC in control (81.4 ± 3.2 μm^2) follicles. Combined with our previous results, these data suggest GnRH-II and its receptor are critical to porcine TC function within preovulatory follicles. Supported by USDA/NIFA AFRI EWD predoctoral fellowship (2022-11301) and Hatch Multistate (NEB-26-244) funds. *USDA is an equal opportunity provider, employer and lender.*