NUTRITIONAL STRESS DURING EARLY SECONDARY OOCYTE GROWTH INDUCES FOLLICULAR ATRESIA AND CHANGES IN OVARIAN GENE EXPRESSION IN COHO SALMON

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Background:

Environmental stressors (e.g., reduced food availability, endocrine disrupting chemicals) are known to disrupt endocrine function in fishes, potentially delaying the age of puberty and affecting gamete quality. Recent experiments in our lab have revealed that prolonged fasting of previtellogenic coho salmon (*Oncorhynchus kisutch*) impairs endocrine function and increases follicular atresia [1]. However, little is known about the molecular processes in the ovary that either regulate atresia or are consequences of atresia. We hypothesized that a nutritional stress that perturbs normal ovarian growth and induces follicular atresia would alter the pattern of gene expression in the gonads. Indeed, in a recent fasting experiment we found that expression of some steroidogenesis-related genes (e.g., *steroidogenic acute regulatory protein*) decreased in fasted salmon, while expression of apoptosis-related genes (e.g., *caspase 3*, *caspase 8 and fas-associated death domain*) were elevated in fasted fish relative to fed fish [1]. The primary aim of this study was to identify additional gene transcripts that are up- or down-regulated in the previtellogenic salmon ovary when ovarian growth is impaired by prolonged fasting. A second goal of the study was to identify candidate genes that might serve as molecular markers of the initiation of atresia.

Methods:

Two year-old coho salmon with ovarian follicles at the late cortical alveolus stage were either fasted or normally fed over a 17-week period starting in March, approximately 10 months before spawning. Fish were subsampled at the initiation of the experiment, and at 3, 9, 14, and 17 weeks after fasting was initiated. The effect of prolonged fasting on previtellogenic follicle growth was monitored by assessing changes in the cellular morphology of ovarian follicles and changes in plasma levels of follicle-stimulating hormone (FSH), estradiol-17beta (E2) and insulin-like growth factor 1 (IGF1), and pituitary FSH content. Reciprocal suppression subtractive hybridization (SSH) cDNA libraries were generated using ovaries from fed and fasted animals collected at week 14. This time point was selected for SSH because it was the earliest time point where fasted salmon showed significantly lower plasma E2 levels and pituitary FSH content, and a higher proportion of follicles either delayed in development or initiating atresia relative to the main cohort of follicles in the ovary. Yet, the stages of the main cohort of follicles were the same in fed and fasted fish at week-14. Therefore, any differences in gene expression at this time point would reflect the effects of disruption of the FSH-ovary axis and atresia onset rather than major differences in development of the main cohort. A total of 480 clones were sequenced per library and annotated by aligning them to the NCBI nr and nt databases. The differential expression of genes identified by SSH was confirmed with quantitative PCR (qPCR) and genes that showed differential expression were analyzed in samples taken over the complete time course of the study (0-17 weeks).

Results and Discussion:

Prolonged fasting reduced body and ovary weight, and increased the proportion of follicles that were either atretic or delayed in stage relative to follicles in normally fed controls. Endocrine analyses showed that fasting reduced plasma IGF1, E2, and pituitary, but not plasma, levels of FSH. The SSH library representing genes up-regulated in the ovary of fasted fish contained genes associated with apoptosis (*programmed cell death protein 4*; *pdcd4*, *lipopolysaccharide-induced TNF factor* and *kruppel-like factor 6*), cortical alveoli components (e.g., *alveolin and serum lectin isoform 2*) and zona pellucida glycoproteins (e.g., *zona pellucida protein X*). The up-regulation of these suites of genes was likely associated with the initiation of atresia or reduced rate of follicle development. On the other hand, the library representing genes up-regulated in the fed ovary contained steroidogenesis-related genes (e.g., *3beta-hydroxysteroid dehydrogenase*; *hsd3b* and *P450 aromatase; cyp19a1a*), TGF-beta superfamily members (*anti-Mullerian hormone*; *amh* and *inhibin alpha; inha*) and cytoskeleton formation-related genes (e.g., *keratin 8*; *krt8*). Thus, genes up-regulated in fed fish were associated with steroid production, cell proliferation and differentiation, and ovarian epithelialization.

Interestingly, ovarian mRNA levels for *hsd3b*, *amh*, *krt8*, and *pdcd4* were significantly different between fed and fasted fish by week 9, before plasma steroid levels and histological differences in ovarian follicle stage were observed. Identification of additional genes from the SSH libraries that are differentially expressed in normally developing versus regressing previtellogenic follicles is ongoing.

Conclusion:

Nutritional stress impaired endocrine function, induced atresia and changed the pattern of genes expression associated with multiple molecular events in coho salmon previtellogenic gonads. This study identified ovarian genes involved in normal previtellogenic oocyte growth as well as those affected by nutritional stress and poor body growth. Genes that were differentially expressed before histological signs of atresia were apparent might be useful markers of early stages of atresia for monitoring impacts of a variety of environmental stressors on ovarian follicle health.

References:

[1]YAMAMOTO, Y., LUCKENBACH, J.A., GOETZ, F.W., YOUNG, G. and SWANSON, P. 2011. Disruption of the salmon reproductive endocrine axis through prolonged nutritional stress: changes in circulating hormone levels and ovarian genes involved in steroidogenesis and apoptosis. Gen Comp Endocrinol.,doi:10.1016/j.ygcen.2011.03.017.