Entering the Twilight Zona:
Exploring the Role of UCHL1 in Regulating Oocyte Proteostasis

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Depletion of the ovarian reserve directly affects female procreative potential and leads to an altered endocrine environment that negatively impacts ovarian and extragonadal health. As the female reproductive system ages faster than any other organ systems in the human body, it is of the utmost importance to understand the specific mechanisms which underlie ovarian aging and promote efforts to preserve the ovarian reserve which is critical for female fertility, longevity and quality of life.

Towards this goal, our laboratory has identified Ubiquitin C-Terminal Hydrolase L1 (UCHL1) as highly enriched in mouse and human oocytes throughout the reproductive lifespan and have found that female mice lacking UCHL1 exhibit severe subfertility, morphologically abnormal oocytes, altered folliculogenesis, and systemic endocrine disruption. We have observed that loss of UCHL1 results in increased rates of granulosa cell (GC) apoptosis and a thickened appearance of the oocyte's zona pellucida (ZP), which has been associated with poor oocyte quality, poor IVF outcomes in humans, and observed in women of advanced maternal age. The formation of the ZP plays an important role in the bidirectional communication between the oocytes and their surrounding somatic GCs, particularly via the connexin proteins of the gap junctions, with disruption resulting in impaired follicle development and female infertility in the mouse. Critically, this communication is also required for ovarian hormone production, specifically estradiol and progesterone, whose serum concentrations directly affect function of the hypothalamic-pituitary-gonadal axis. Our lab has observed increased levels of Zona Pellucida Protein 3 (ZP3) expression in UCHL1 KO oocytes, although differential expression of ZP3 is not observed at the mRNA level, suggesting that this phenotype is a result of altered protein turnover. We have also detected a change in the organizational pattern of Connexin 37, the predominant connexin in the oocyte, suggesting that in the absence of UCHL1, gap junctions may not properly form.

To better understand molecular mechanisms of UCHL1 function in the mammalian oocyte, we performed a pilot proteomic analysis on oocytes isolated from UCHL1 KO and WT mice, finding significant dysregulation of over 1000 peptides, including ZP3 and Zona Pellucida Protein 2, indicating altered oocyte proteostasis in these animals. Interestingly, UCHL1 is a Ubiquitin Proteasome Pathway (UPP) regulator which is known to maintain the cellular balance of monoubiquitin and thus regulate protein turnover. Currently, no current studies are investigating the molecular underpinnings of the UPP in the context of ovarian aging.

While our exciting findings point to an essential role of UCHL1 and the ovarian UPP in the long-term functioning of the mammalian ovary, our previous work has been limited by the neurological aging phenotypes of full UCHL1 KO mice, which are not able to live past six

months of age due to rapid motor neuron decline. Furthermore, interpretation of these results has been confounded by the intersection of functions of the neuroendocrine tissues in which UCHL1 is also expressed. To circumvent these obstacles, and to understand the oocyte-specific roles of the UPP in reproductive longevity, we have generated an oocyte-specific UCHL1 KO mouse line which will help elucidate the effect of oocyte-specific loss of UCHL1 on long-term fertility, fecundity, ovarian aging and proteostasis. This research will illuminate the molecular mechanisms which underlie oocyte quality and ovarian aging with the potential to inform our understanding of idiopathic female infertility, reproductive outcomes, and overall women's health.