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BECN1, corpus luteum function, and preterm labor

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Progesterone is a steroid hormone that is necessary to maintain pregnancy in mammals. We recently found that mice with a conditional deletion of *Becn1/Beclin 1* specifically in the progesterone-synthesizing cells of the corpus luteum, had reduced progesterone synthesis and these mice failed to maintain pregnancy.[1] Furthermore, we identified that lipid storage and feedback through PRLR (prolactin receptor) and LHCGR (luteinizing hormone/choriogonadotropin receptor) were negatively affected by *Becn1* deletion. BECN1 is necessary for the interaction of the 2 catalytic subunits of the class III phosphatidylinositol 3-kinase complex, PIK3C3, and PIK3R4, which are responsible for the generation of phosphatidylinositol 3-phosphate that is required for nucleation of the phagophore. Work from Sun et al. and Itakura et al. demonstrated that this BECN1 complex is also necessary for the fusion of autophagosomes and endosomes with lysosomes. Therefore, we suspected that ablating *Becn1* in luteal cells would inhibit macroautophagy, hereafter referred to as autophagy. In support, we provide evidence that autophagic flux is reduced in our model. Thus, this study provides evidence that *Becn1* is necessary for steroid production in murine luteal cells.

Keywords: Beclin 1, corpus luteum, pregnancy, preterm birth, steroidogenesis

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Preterm birth, defined as parturition at less than 37 wk of gestation, affects an estimated one in 8 human pregnancies. In addition to being the leading cause of neonatal death worldwide, preterm birth is also directly associated with lifelong adverse health. The events leading to preterm birth are thought to be multifactorial; however, the etiology is not completely understood. Approximately 50% of all preterm births are classified as idiopathic. Other leading causes are the premature rupture of the placental membrane and elective preterm deliveries. In the study of preterm birth, progesterone has proven a viable treatment for high-risk individuals but the mechanism of action is poorly understood. Although, we do know that circulating levels of biologically active progesterone in mothers of full-term births remains high until hours before parturition. The source of progesterone is 2-staged, in humans, with the first 9 wk coming from the steroid-producing luteal cells of the corpus luteum, an ephemeral endocrine gland formed from the ovarian follicle post-ovulation. After 9 wk of gestation, the placental trophoblast takes over synthesis for the remainder of gestation. Since 2 different cell types located in 2 different tissues are responsible for progesterone synthesis, it remains difficult to study the processes underlying progesterone synthesis in humans. In the mouse, however, the corpus luteum solely produces progesterone to maintain pregnancy. This concept makes the mouse an ideal model to study how the corpus luteum function and progesterone synthesis interact to determine the length of gestation.

In our previous work, we showed that *Becn1* and *Atg7* are independently necessary for producing the oocyte reserve in perinatal mouse ovaries, suggesting that autophagy is necessary for perinatal oocyte survival. Several other studies over the past decade have implicated both BECN1 and autophagy in the function and survival of the corpus luteum; however, targeted deletion of genes encoding key pathway members have not been performed. In our current experimental design, we used a conditional knockout approach to ablate *Becn1* specifically in granulosa cells of the developing ovarian follicle, which terminally differentiate into

luteal cells, in order to determine if autophagy is necessary for corpus luteum formation and function.

The first result was that corpora lutea were present in *Becn1* flox/flox conditional knockout ovaries at pregnancy d (P) 8.5, which suggested that *Becn1* is not necessary for corpus luteum formation. Next, pregnant, conditional-knockout dams began parturition up to 4 d earlier than wild-type siblings. We confirmed that there was a reduction in circulating progesterone after P13.5 by quantifying progesterone throughout pregnancy. The variance in day of parturition in the conditional knockout females was high, suggesting that the amount of gene knockout was variable and incomplete. Using a more complete knockout strategy, females with one floxed allele and one null allele were evaluated; none of these conditional knockout females gave birth, and resorption occurred between P5.5 and P14.5. The dependency on progesterone for the failure in pregnancy was confirmed when the gestation length of both conditional knockout models was rescued to wild-type length with exogenous progesterone treatments. Since progesterone is synthesized from cholesterol, we performed neutral lipid analysis on histology sections and observed that *Becn1* conditional knockout corpora lutea have reduced lipid stores compared to wild-type luteal cells. As expected from ablating *Becn1,* we saw an increase in protein quantities of SQSTM1 and LC3, suggesting that the flux of autophagy is reduced in conditional knockouts. Of particular interest was our observation that breeding our mice to GFP-LC3 mice allowed us to detect numerous GFP puncta within the luteal cells. Transmission electron microscopy further revealed abundant empty vacuoles and autophagosomes in conditional knockout luteal cells that were not present in wild-type luteal cells. This suggested that the function of *Becn1* in luteal cells biases toward promoting the fusion of endosomes and autophagosomes to lysosomes versus autophagosome formation.

From an experimental point of view, this work further substantiates the notion that BECN1 participates in more processes than just autophagy. Further work needs to be completed to characterize whether the phenotype we have uncovered is an autophagy-specific mechanism of BECN1. However, the fact that we saw autophagosomes and GFP-LC3 puncta accumulating in the conditional knockout cells suggest that autophagy plays a significant role in luteal cells. On the other hand, we show an excess of empty vesicles that are likely endosomes, and the positive feedback from activation of PRLR and LHCGR is reduced by *Becn1* ablation. This provides a hypothesis that *Becn1* is necessary for PRLR and LHCGR turnover. Furthermore, reduced lipid storage could be due to the inability to recycle LDL receptors that could be present in the empty vesicles in our model. Li et al. showed that BECN1 is directly associated with testosterone production in testicular Leydig cells. We provide data showing a second steroidogenic cell that relies on the function of BECN1 to produce a steroid hormone. Overall, we identify a new preterm labor model that uncovers *Becn1* as a necessary factor for progesterone production, and it will be important to determine if *Becn1*-dependent steroidogenesis is specific to Leydig and luteal cells or conserved among all steroid-producing cells.

Disclosure of Potential Conflicts of Interest

No potential conflicts of interest were disclosed.

Reference

1. Gawriluk, T. R., Ko, C., Hong, X., Christenson, L. K., & Rucker, E. B. (2014). Beclin 1 deficiency in the murine ovary results in the reduction of progesterone production to promote preterm labor. Proceedings of the National Academy of Sciences, 201409323. In Press as PNAS Early Edition.