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RAPAMYCIN PARTIALLY RESCUES OOCYTE DYSFUNCTION IN MICE DEFICIENT FOR MITOCHONDRIAL STRESS RESPONSE PROTEIN CLPP.

T. Wang,a E. Babayev,b Z. Jiang,c M. Zhang,a E. Esencan,a E. Seli.a,d aOB&GYN, Yale School of Medicine, New Haven, CT; bFeinberg School of Medicine, Northwestern University, Chicago, IL; cLouisiana State University, Baton Rouge, LA; dIVIRMA New Jersey, Basking Ridge, NJ.

OBJECTIVE: CLPP (caseinolytic peptidase P) is a key regulatory protein for mitochondrial unfolded protein response (mtUPR) and helps maintain homeostasis in response to metabolic and cellular stress. CLPP is required for oocyte and early embryo development, and global germline knockout of Clpp results in female infertility and accelerated follicular depletion, associated with mTOR pathway activation. The aim of the current study was to determine whether rapamycin, a known inhibitor of mTOR pathway, could rescue oocyte competence in Clpp-knockout (Clpp-/-) mice.

DESIGN: Experimental study.

MATERIALS AND METHODS: Rapamycin rescue experiments were performed in vivo (2mg/kg rapamycin or saline injected intraperitoneally daily for 14 days) and in vitro (1 nM rapamycin added to the culture medium vs media alone). Clpp-/- mice/oocytes treated with rapamycin [KO-RAP] were compared to untreated Clpp-/- mice/oocytes [KO-CON] and to wild type [WT]. Western blotting (WB) and immunofluorescence (IF) were used to determine protein expression in ovaries and oocytes, respectively. Ability to generate germinal vesicle (GV) and metaphase II (MII) oocytes was assessed after injection with PMSG (5IU) or PMSG and hCG (5IU), respectively. Spindle morphology was determined by staining with a-tubulin and DAPI. ANOVA, student's t-test, and Chi Square analysis were used for statistical analysis as appropriate.

RESULTS: KO-RAP mice produced significantly higher number of GV (20.3 _ 3 vs 15 _ 2.6%, $p<0.05$) and MII (13.3 _ 2.5 vs 6.5 _ 1.3%, $p<0.05$) oocytes compared to KO-CON. In addition, GV oocytes obtained from KO-RAP mice showed higher germinal vesicle breakdown (GVBD) (60 _ 8.7 vs 24.5 _ 9.2%, $p<0.05$) and normal spindle formation (60 _ 17.4% vs. 24.5 _ 18.4%, $p<0.05$) rates compared to KO-CON, although lower than that observed in WT (97.5_2.5% GVBD, 97.5_5% spindle formation, both $p<0.001$). In vitro rapamycin treatment also resulted in improved GVBD (69 _ 5.13 vs 39.33_ 8.54%, $p<0.05$), and normal spindle rates (56.84_8.73 vs 30.71_5.68%, $p<0.01$). mTOR pathway downstream regulatory proteins (p-S6, p-S6K, p-4EBP1, p-AKT473, p-mTOR2481) detected by WB were significantly up-regulated in Clpp-/- ovaries ($p< 0.05$). Similarly, IF staining showed p-S6 and p-AKT473 expression to be higher in Clpp-/- GV oocytes ($p < 0.001$). After in vivo rapamycin treatment, p- S6, p-S6K, p-4EBP1, p-AKT473, and p-

mTOR2481 expression were significantly decreased in KO-RAP ovaries compared to KO-CON, confirming mTOR pathway suppression in response to rapamycin.

CONCLUSIONS: Our findings demonstrate that rapamycin can partially rescue the reproductive dysfunction in Clpp-/- oocytes by suppressing mTOR pathway. The potential benefit from rapamycin treatment in other mitochondrial dysfunction models and potentially in human subfertility remain to be investigated.