Title: DOES RAPAMYCIN DELAY OVARIAN AGING AND DECREASE SENESCENCE? A FIRST-EVER ANALYSIS IN A NON-HUMAN PRIMATE MODEL

OBJECTIVE: Rapamycin prolongs reproductive lifespan in mice by halting primordial follicle activation. The impact of rapamycin on the preantral follicle pool and senescence markers during ovarian aging in macaques was evaluated.

MATERIALS AND METHODS: One ovary was removed from young (n=2, 6–9 yr) and old (n=2, 17–21 yr) adult female rhesus macaques during a normal menstrual cycle (pre-treatment). The remaining ovary was obtained after animals were treated with rapamycin (bid, IM, 0.02mg/kg) for 10 months. Ovaries were fixed and serially sectioned for follicle counting (each 10th section, 15-39 sections/ovary). Immunohistochemical analyses were performed for anti-Mullerian hormone (AMH) and cellular senescence markers p16, p53, and p21 (1 slide/ovary). Qualitative comparisons were made due to the small sample size.

RESULTS: The primordial follicle pool was decreased in young (3,939 pre-treatment vs. 2,219 post-treatment), but similar in old (555 pre- vs. 574 post-treatment) females after rapamycin. The number of transitional primordial follicles was greater before rapamycin than after in both young (14,920 vs. 4,924) and old (1,915 vs. 1,311) females. The number of primary follicles before (2,617) rapamycin was greater than after (560) in young and old females (518 pre- vs. 428 post-treatment). A similar proportion of follicles positive for p16 was seen before and after rapamycin in both young and old females. Similar findings were also observed for AMH, except there are fewer positive follicles in the rapamycin-treated older group. The proportion of follicles staining positive for both p53 and p21 was increased in both young and old monkeys after treatment.

CONCLUSIONS: Rapamycin had no impact on the primordial and primary follicle pools in old female macaques while unexpectedly decreasing both pools in young females. While the number of p16-positive follicles was unaffected by rapamycin treatment, the number of p53 and p21-positive follicles was increased by treatment in both age cohorts.

IMPACT STATEMENT: At the dose and treatment interval used, rapamycin does not appear to suppress follicular activation and has mixed effects on senescence markers in aging nonhuman primate ovaries.

	Pre-Treatment		Post Treatment	
	Young	**Old**	**Young**	**Old**
Primordial	3,939	555	2,219	574
Transitional primordial	14,920	1,915	4,924	1,311
Primary	2,617	518	560	428
Transitional primary	800	510	348	411
Secondary	171	219	90	94
Multi-layer	601	568	495	666
Abnormal morphologic features	109	137	181	149

Antral	79	73	92	54
Atretic antral	28	13	36	6
Multi-oocytic	521	55	42	57
Unknown	6	27	27	54
Corpus luteum	1	1	1	1
Total	23,792	4,591	9,015	3,805
Primordial (%)	17	12	25	15
Transitional primordial (%)	63	42	55	34
Primary (%)	11	11	6	11
p16 (%)	0.9	0.3	0.5	0
p53 (%)	0.7	8	6	20
p21 (%)	4	10	11	22
AMH (%)	91	95	86	86