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Does Rapamycin Delay Ovarian Aging and Decrease Senescence? A First Ever Analysis in a Non-Human Primate Model

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PURPOSE & OBJECTIVES

Cellular senescence, which entails a terminal state of growth arrest, increases with age. While there isn't a single marker for senescence, p16, p53 and p21 are frequently used to detect senescence as they are components of the DNA damage pathway that regulate cell cycle arrest. Additionally, anti-Mullerian hormone (AMH), a marker of ovarian reserve, has been used as an indicator of primordial follicle activation. Localization of these markers by immunohistochemistry (IHC) can be used to evaluate the extent of senescence and follicular activation in an aging ovary.

Rapamycin, a mammalian target of rapamycin [mTOR] inhibitor, is used as an immunosuppressant and is also of interest as an anti-aging intervention. In mice, rapamycin prolongs reproductive lifespan by halting primordial follicle activation. The influence of rapamycin in nonhuman primates has yet to be investigated.

Our objective was to evaluate the effect of rapamycin on the preantral follicle pool and ovarian senescence by comparing follicle numbers as well as p16, p53, p21 and AMH markers in female rhesus macaques.

MATERIAL & METHODS

- One ovary was removed from young (n=2, 6–9 years) and old (n=2, 17–21 years) adult female rhesus macaques (pre-treatment).
- Animals were treated with rapamycin (bid, IM, 0.02mg/kg) for 10 months. After treatment, the remaining ovary was obtained. Ovaries were fixed and serially sectioned at 5th micron. Every 10th slide was stained with hematoxylin and eosin (H&E) to evaluate ovarian tissue morphology for follicle counting.
- IHC was performed for AMH and cellular senescence markers p16, p53, and p21 (1 slide/ovary). Qualitative comparisons were made due to the small sample size.

At the dose and duration used, rapamycin **did not increase but maintained** the ovarian reserve of primordial follicles in old macaques.

Unexpectedly, rapamycin **decreased** the primordial follicle pool in young monkeys, suggesting a deleterious effect on ovarian reserve in young females.

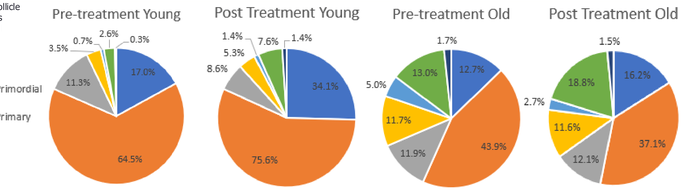
Cells expressing the senescence markers p16, p53 and p21 were **undetectable or ≤1% in preantral follicles** regardless of age or treatment.

AMH expression was lower in older females due to the decline in the preantral follicle pool that occurs with aging.

These studies provide a framework to further evaluate the optimal duration and age of intervention required to extend reproductive lifespan in nonhuman primates with future application in women.

RESULTS

Pie Chart: H&E Follicle Count Proportions in Young and Old Monkeys



Tables 1 & 2: Total Follicle Counts in Young and Old Monkeys from H&E-stained serial sections

	Young	
	Pretreatment	Post treatment
Primordial	3,939	2,219
Transitional Primordial	14,920	4,924
Primary	2,617	560
Transitional Primary	800	348
Secondary	171	90
Multilayer	601	495
AMF	109	181
Antral	79	92
Atretic Antral	28	36
Multi-oocytic	521	42
Unknown	6	27
Corpus Luteum	1	1

	Old	
	Pretreatment	Post treatment
Primordial	555	574
Transitional Primordial	1,915	1,311
Primary	518	428
Transitional Primary	510	411
Secondary	219	94
Multilayer	568	666
AMF	137	149
Antral	73	54
Atretic Antral	13	6
Multi-oocytic	55	57
Unknown	27	54
Corpus Luteum	1	1

Table 3: IHC Follicle Counts

Percentage of Positive Preantral Follicles by Age Group				
	Pre-treatment		Post treatment	
	Young	Old	Young	Old
p16 (%)	0	0	0	0.4
p53 (%)	0	0	0	0
p21 (%)	0.2	0.9	1	1
AMH (%)	74	32	63	34

Insets = NSB

Figure 1: p16 positive, young, post-treatment, 20x (arrow)

Figure 2: p53 positive, young, post-treatment, 20x (arrow)

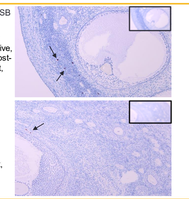
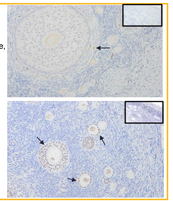


Figure 3: p21 positive, old, pre-treatment, 20x (arrow)

Figure 4: AMH positive, young, post-treatment, 20x (arrow)



RESULTS

- After rapamycin treatment, the primordial follicle pool decreased in the young females but remained similar in the old females.
- The number of transitional primordial follicles was greater before rapamycin than after in both young and old females. The number of primary follicles was greater before rapamycin treatment than after in young and old females.
- Essentially no preantral follicles were positive for p16 or p53 before or after rapamycin treatment in both young and old females. However, rare ovarian stromal cells were positive for p16 and p53 in the older females both pre- and post-treatment.
- There were more preantral follicles positive for p21 after rapamycin treatment in young females. There was a similar proportion of positive follicles in the older group. p21 was primarily found in the theca cells of the preantral follicles regardless of age or treatment.
- The percentage of AMH positive preantral follicles after rapamycin in young females decreased, but a similar number of preantral follicles were positive in the older females before and after treatment.

CONCLUSIONS

- In contrast to mice, rapamycin did not increase the ovarian reserve of primordial follicles. Young monkeys may experience a depletion of their ovarian reserve in response to rapamycin treatment. Rapamycin, at the dose and duration used, did not show utility for extending reproductive lifespan in macaques.
- While no preantral follicles were positive for p53 and few were positive for p16, the number of p21-positive follicles increased while AMH decreased after rapamycin in young females. This indicates that senescent markers may not be reliable indicators of aging in the non-human primate model, but positive AMH cells accurately reflect the preantral follicle pool.

REFERENCES

ACKNOWLEDGEMENTS

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