



# The Effect of Aging on Peripheral Blood Mononuclear Cell Proliferation in Labrador Retrievers

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## Objective

It is well documented that companion animals are living longer and as animals age their cells undergo immuno-aging or senescence, an age associated process of deterioration. In an effort to observe how aging plays a role in immune cell proliferation, twelve healthy Labrador retrievers (6-Young, 3Male/3Female, 6-Senior, 3Male/3Female) were selected to participate in a study to determine what effect aging may have on the proliferative rate of peripheral blood mononuclear cells (PBMCs). PBMC telomere length has been correlated with average lifespan in 15 breeds and data shows dogs lose telomeric DNA ~10-fold faster than humans.<sup>1</sup>

## Methods

### Animals & Housing

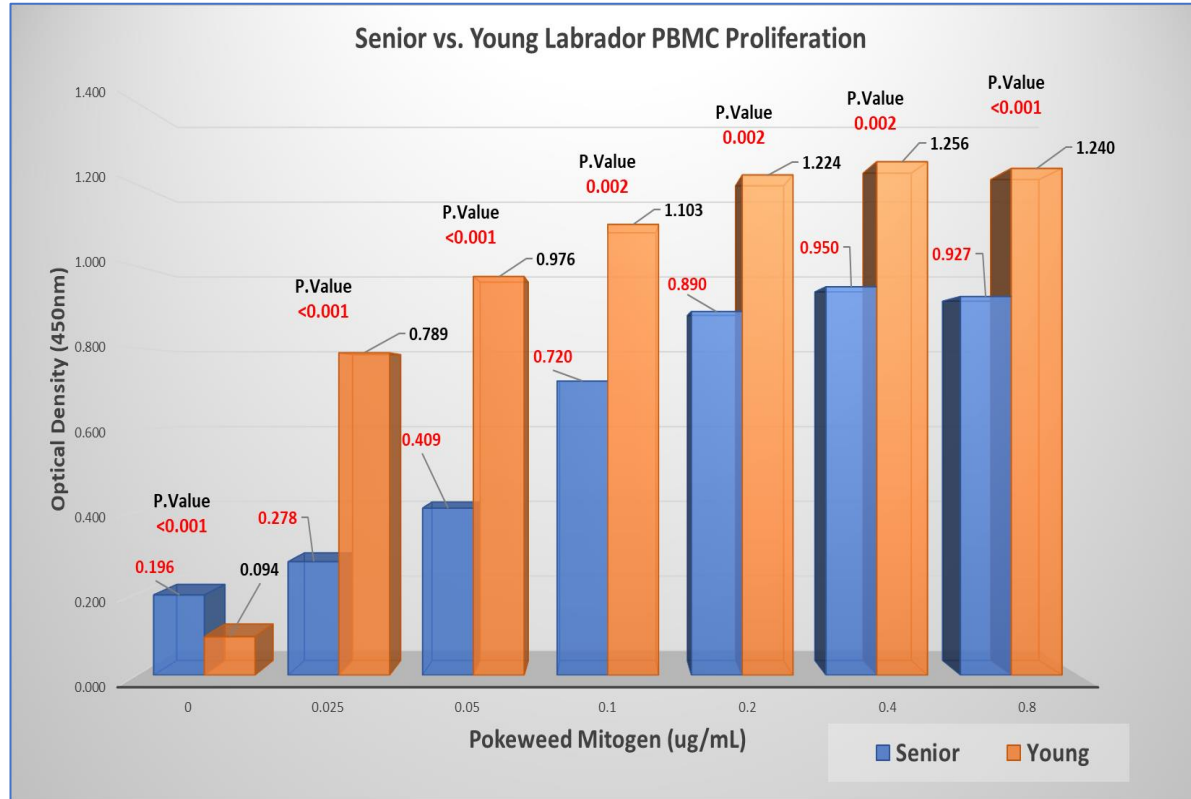
- All dogs selected from colony of Labrador Retrievers at Four Rivers Kennel.
- All dogs housed in controlled kennel environment and were aired outside in social groups for approximately six hours daily and kenneled individually overnight.
- Water ad libitum

### Diet & Treatment

- All dogs were fed the same diet, amounts determined by body weight.
- Dogs were equally divided into two treatment groups based on age, sex, body weight, and genetic lineage.
- Mean Age: *Young dogs* – 2.38±0.11 yrs.; *Senior dogs* – 8.74±0.11 yrs. (P.Value <0.001)

### Blood Collection, PBMC Isolation, Counting & Plating

- 4 mL of whole blood was collected in Lithium-Heparin vacutainer tubes via cephalic venipuncture.
- PBMCs were isolated using Lymphoprep™ density gradient in 15 mL SepMate™ isolation conical tubes (Stemcell Technologies).
- Cells washed twice, dPBS (2% FBS).
- Cells dyed using 0.4% Trypan Blue stain & counted via Invitrogen Countess II automated counter (ThermoFisher).
- Cells seeded at 5x10<sup>4</sup> in 96-well microplates.



### Culture & Proliferation

- Cells cultured in RPMI-1640 complete medium (5% FBS), and mitogenic stimulation via 2-fold serial dilutions of *Phytolacca Americana* lectin (pokeweed mitogen-PWM) ranging from 0.8 - 0.025 µg/mL.
- Following 48hr incubation in 5% CO<sub>2</sub>/95% humidity, 100µM Bromodeoxyuridine (5-bromo-2'-deoxyuridine, BrdU; a synthetic analog nucleoside of thymidine), was added to each culture well and further incubated 24hrs to allow DNA incorporation.
- Cells were harvested, denatured, and BrdU incorporated in newly synthesized DNA was bound by monoclonal antibodies (anti-BrdU-POD). The immune complexes were detected by tetramethyl-benzidine (TMB), reaction stopped with 1M Sulfuric acid (H<sub>2</sub>SO<sub>4</sub>), and quantified by measuring absorbance at 450nm.



## Results

### Proliferative Quantification

- PWM-induced PBMC proliferation was significantly higher in Young dogs than Senior dogs at every concentration (Mean: Senior-0.624±0.02; Young-0.955±0.02 SEM, P.Value <0.001). Rate difference by concentration was as follows:

Total	0 ug/ml	0.025 ug/ml	0.05 ug/ml	0.1 ug/ml	0.2 ug/ml	0.4 ug/ml	0.8ug/ml	AVG
PWM	0.196	0.278	0.409	0.720	0.890	0.950	0.927	0.624
Senior	0.196	0.278	0.409	0.720	0.890	0.950	0.927	0.624
SEM	0.013	0.058	0.068	0.080	0.073	0.066	0.054	0.024
Young	0.094	0.789	0.976	1.103	1.224	1.256	1.240	0.955
SEM	0.013	0.054	0.062	0.079	0.073	0.066	0.054	0.023
P.Value	<0.001	<0.001	<0.001	0.002	0.003	0.002	<0.001	<0.001
Female								
PWM	0.208	0.314	0.429	0.547	0.697	0.833	0.863	0.556
Senior	0.208	0.314	0.429	0.547	0.697	0.833	0.863	0.556
SEM	0.020	0.081	0.097	0.122	0.113	0.102	0.083	0.035
Young	0.111	0.915	1.080	1.179	1.278	1.281	1.270	1.017
SEM	0.016	0.069	0.079	0.100	0.092	0.083	0.068	0.029
P.Value	0.003	<0.001	<0.001	0.002	0.002	0.008	0.003	<0.001
Male								
PWM	0.185	0.241	0.389	0.893	1.083	1.068	0.990	0.693
Senior	0.185	0.241	0.389	0.893	1.083	1.068	0.990	0.693
SEM	0.016	0.081	0.097	0.104	0.092	0.083	0.068	0.031
Young	0.077	0.663	0.871	1.028	1.171	1.232	1.211	0.893
SEM	0.020	0.081	0.097	0.122	0.113	0.102	0.083	0.035
P.Value	<0.001	0.005	0.007	0.042	0.025	0.599	0.189	<0.001

## Conclusions

These results coincide with other published literature and this data suggests age plays a critical role in immunity and proliferative ability of PBMCs in the Labrador retriever. Further studies could include assaying cytokine activity<sup>2</sup> and counting sub-populations using flow cytometry for a better look at cellular demographics based on age.

## References

- <sup>1</sup>Fick, L. J., Fick, G. H., Li, Z., Cao, E., Bao, B., Heffelfinger, D., ... Riabowol, K. (2012). *Telomere Length Correlates with Life Span of Dog Breeds. Cell Reports, 2(6), 1530–1536.* doi:10.1016/j.celrep.2012.11.021
- <sup>2</sup>Leng, S. X., Yang, H., & Walston, J. D. (2004). *Decreased cell proliferation and altered cytokine production in frail older adults. Aging Clinical and Experimental Research, 16(3), 249–252.* doi:10.1007/bf03327392