



A role of iNOS gene expression in the anti-inflammatory and tissue protective mechanisms of 905 nm pulsed laser therapy

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Rational for interrogating NO

- Low levels of NO production are protecting an organ from ischemic damage.
- Chronic expression of NO is associated with carcinomas and inflammatory conditions (juvenile diabetes, multiple sclerosis, arthritis and ulcerative colitis)

iNOS in previous LLLT studies.

- Yang et al. showed that 632 nm exposure suppressed amyloid β -peptide induced expression of IL-1 β and iNOS in astrocytes (*Neuroscience* 171 2010).
- Abdel et al. noted a 50% increase of iNOS mRNA following 632 nm exposure in normal muscle but ~150% increase in Duchenne muscular dystrophy (*Acta Myol* 26 2007)
- 780 nm mediated LLLT of macrophages could increase the iNOS expression so at high lipopolysaccharide stimulation the effect is lost. (*Laser Surg Med* 40 2008)

Motivation and Approach

- Determine the action spectra of iNOS expression modulation following LLLT
 - evaluating the influence of age as a immunomodulator on LLLT mediated iNOS expression
 - impact of high peak power vs. cw exposure on LLLT modulation of iNOS expression.
- Monitoring iNOS expression by production of luciferase being under the same promoter as iNOS gene.
 - Time resolved monitoring of the bioluminescence

Methodology

Injection of Zymosan A: (yeast cell wall) in PBS
FVB/N-Tg(iNOS-luc) mice



Laser irradiation:

15 min after injection

Wavelengths:

635nm (n=5)

660nm (n=8) cw / Pulsed (10 μ sec /1kHz)

690 nm (n=4)

785nm (n=4)

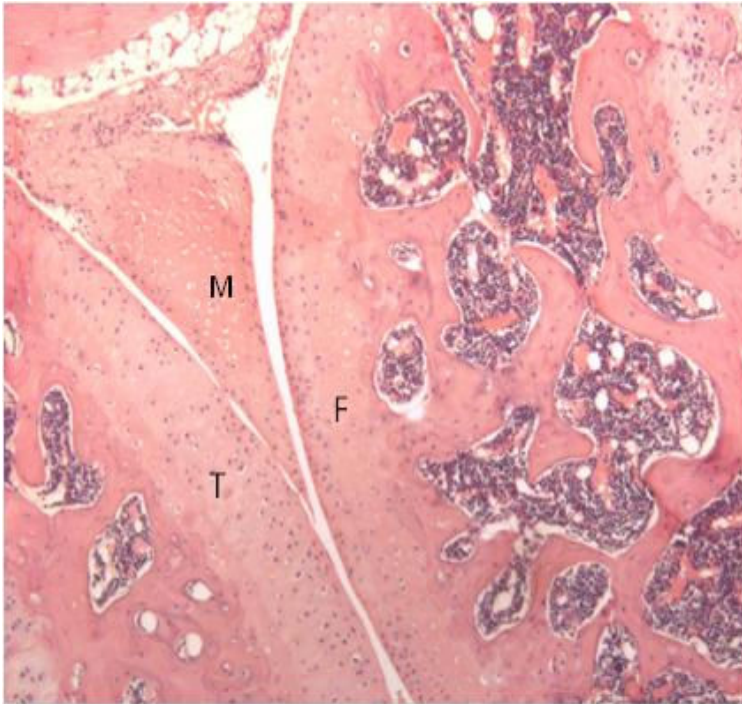
808nm (n=5)

905nm (n=15) cw / Pulsed (200nsec /10 kHz)

Average Irradiance: 50 mW cm⁻²

Radiant Exposure: 5J cm⁻²

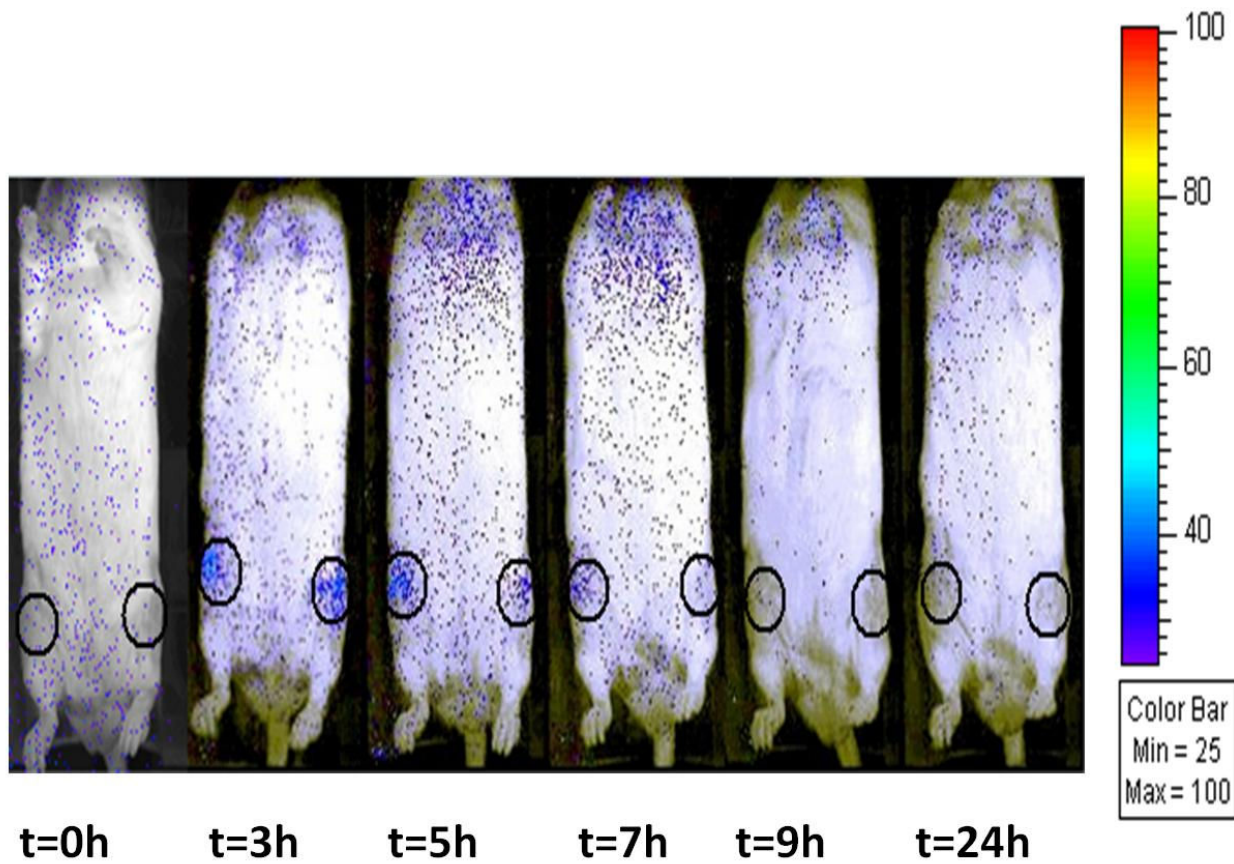
Basic Histology



**Sham inflammation
induction** PBS [5h]

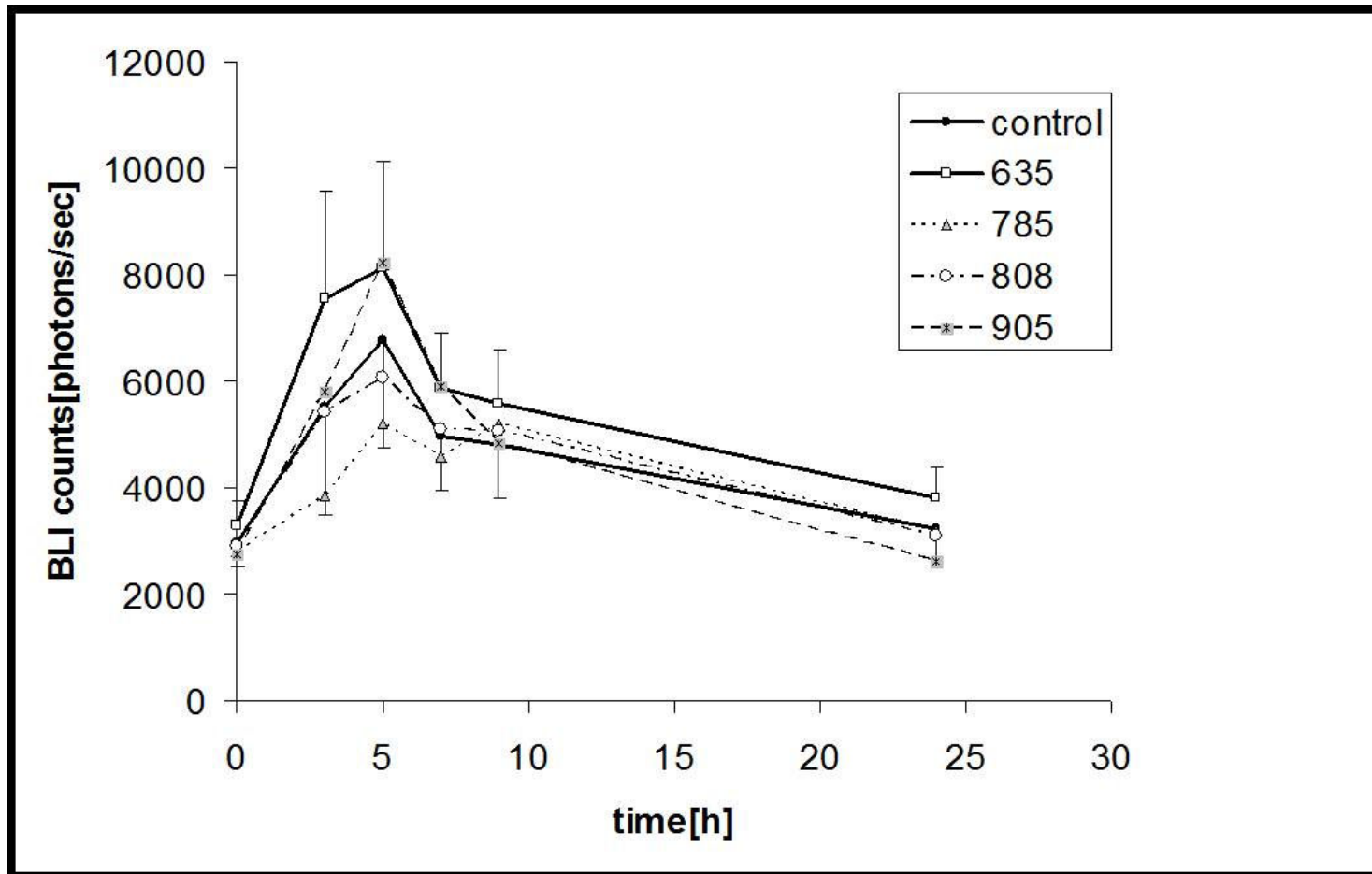
**Zymosan A inflammation
induction** PBS [5h]

Raw BLI images



Zymosan A no LLLT.

BLI time kinetics mice 10-30 weeks old

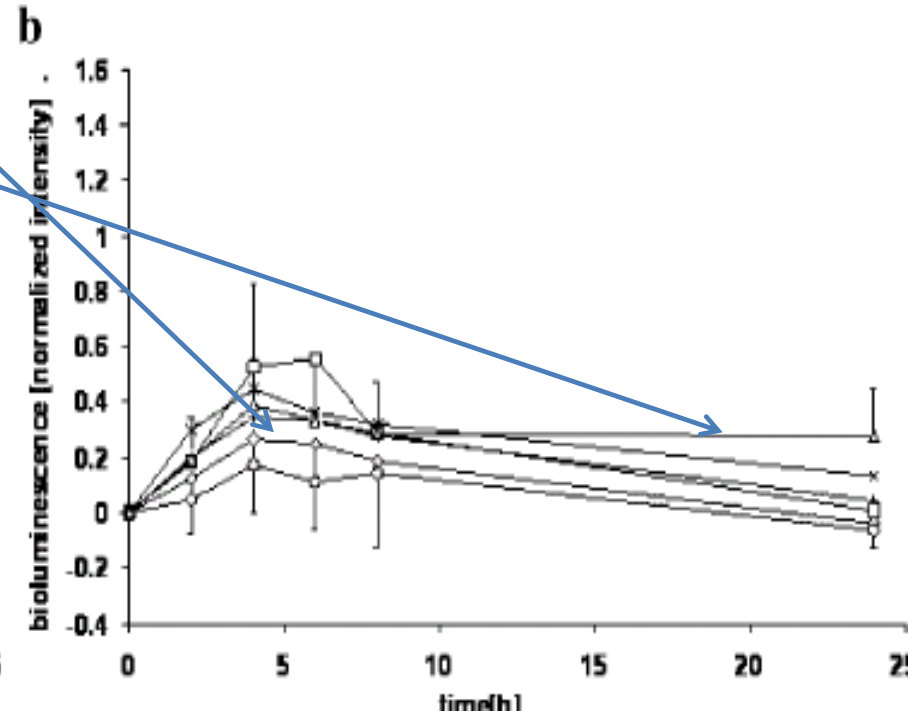
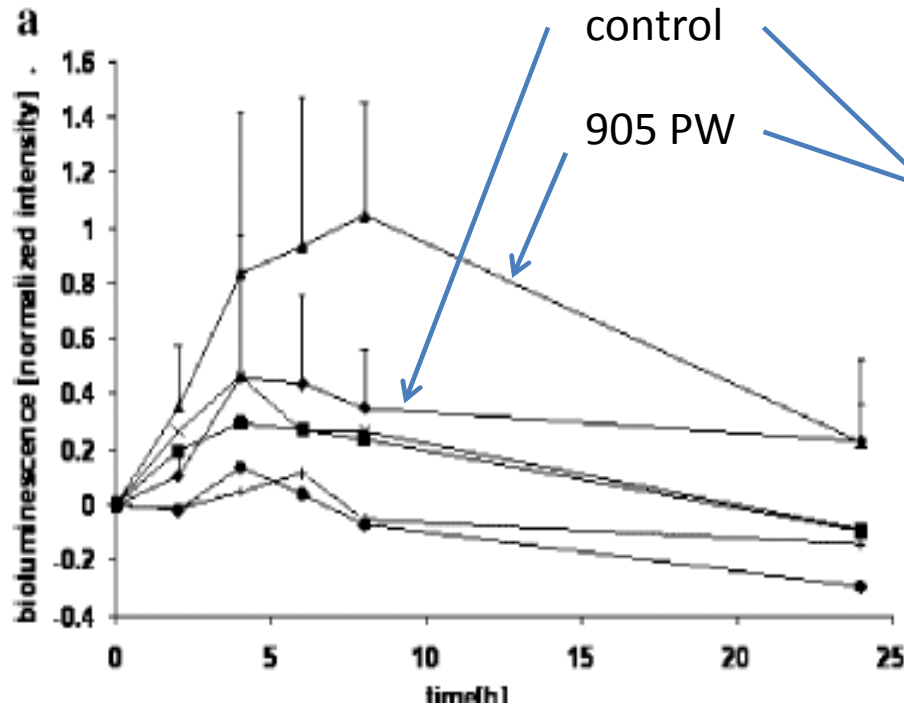


from Moriyama Y. et. al. Photochem. Photobiol. 2005, 81:1351-1365

BLI time kinetics

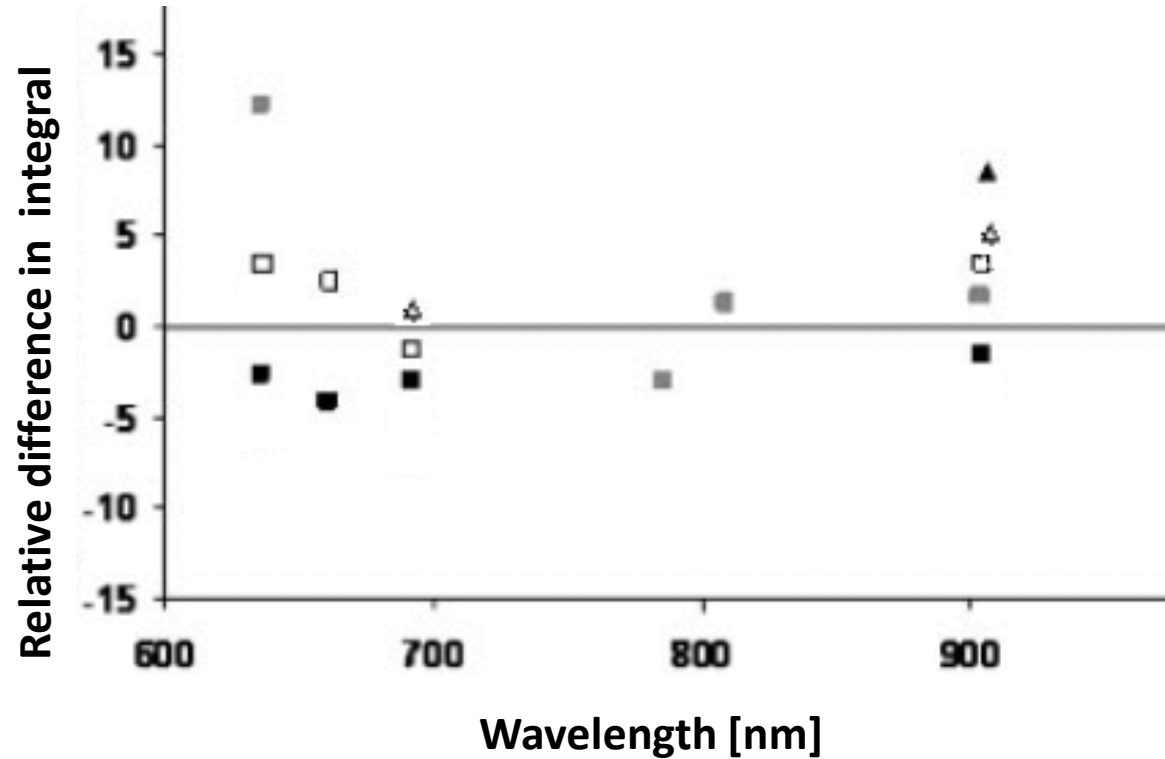
mice <15 weeks

mice >15 weeks



- ◆ controls, +635 nm
- 660 nm (cw and pw)
- 690 nm
- × 905 nm CW
- ▲ 905 nm pulsed wave (PW).

Modulation of iNOS expression over non LLLT-treated animals



Difference is BLI Integral as Function of Wavelength and Ages

Groups	age< 15	15>age<35	35<age
635	-2.1	4.3 ★	11.3★
660	-4.3 ★	3.2	
690	-1.3	-2.3	
780			-2.1
808			0.8
905 CW	-1.8	3.7 ★	2.1 ★
905 PW	6.8 ★	4.6 ★	

P values considered statistically significant are represented with asterisk (*).

Pulsed

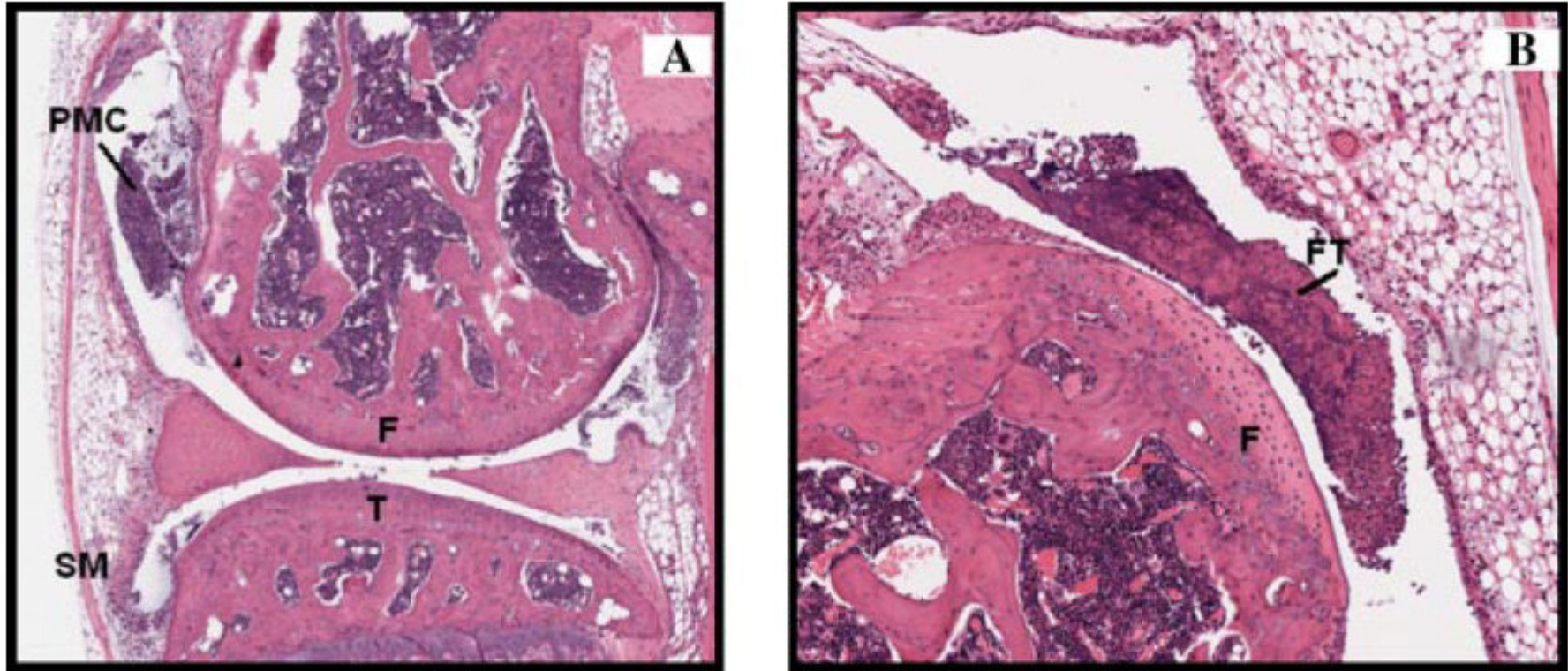


Fig. 3. Histology of knee joints treated with LLLT of 690 nm (A) and 905 nm pulsed wave (B) after 24 hours. (F, femoral condyle; T, tibia; SM, synovial membrane; PMC, polymorphonuclear cells; FT, fibrous tissue).

Discussion

- LLLT 15 minutes post- Zymosan A ↓ recruitment of inflammatory cells for majority of protocols independent of iNOS expression
- LLLT ↑ the expression of iNOS per cell with PW 905 independent of age with an apparently earlier time to peak (~5 hrs).
- With LLLT older animals can exhibit the inflammatory response of a young control animal.
- It seems that iNOS expression is playing a role in the anti-inflammatory mechanisms of LLLT.
- Limitation:
 - long duration of the study with breeding pairs of variant age.
 - Mix of homozygous and heterozygous state
 - Success rates in Zymosan A and Luciferin administration may be variant.
 - Experimental groups not randomize over the duration of these experiments. The number N of animals is still low.

Conclusion

CW appears to have little effect on younger healthy animals.

More LLLT protocols appear effective for old animals.

Pulsed delivery appears to be providing an increased iNOS expression.

Only 905PW appears to influence iNOS expression in young animals.

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