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### The Progesterone Receptor - To Be or Not to Be: The Anti-inflammatory Effects of Progesterone in RAW 264.7 Cells

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Brandon, Christopher I. Jr. and George, Bagie, "The Progesterone Receptor - To Be or Not to Be: The Anti-inflammatory Effects of Progesterone in RAW 264.7 Cells" (2017). *Interdisciplinary STEM Teaching & Learning Conference (2012-2019)*. 5.  
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# The Progesterone Receptor - To Be or Not to Be: The Anti-inflammatory Effects of Progesterone in RAW 264.7 Cells

Christopher Brandon Jr., Ph.D.  
and Bagie George, Ph.D.



# The Inspiration...





# Outline

- Introduction
- Materials and Methods
- Results
- Discussion
- Questions



# Progesterone

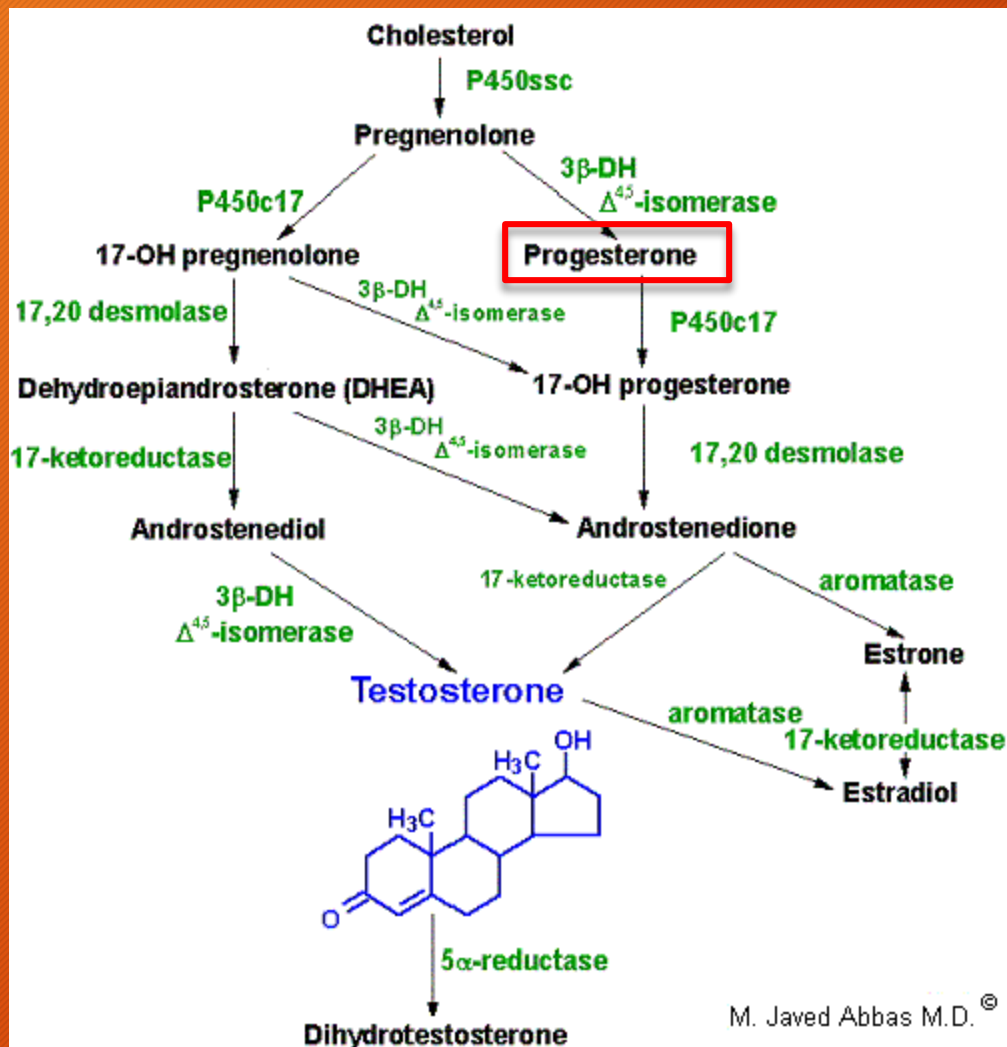


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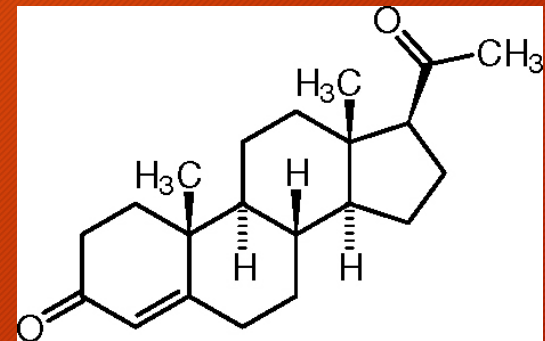
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# Steroid Hormone Biosynthetic Pathway



M. Javed Abbas M.D. ©



# Progesterone Signaling



## Signaling through nuclear receptor

- NR3C3
- Transcription factor

## Signaling through membrane receptor

- G-Protein Coupled Receptor
- Activation intracellular signaling cascades
- Many isoforms: mPR $\alpha$ , mPR $\beta$ , mPR $\gamma$ , mPR $\epsilon$ , mPR $\delta$



# Progesterone Physiology



## Males

- Precursor to testosterone
- Inhibitory gonadotropin action
- Mediator of **capacitation**

## Females

- Predominates during luteal phase
- Active in endometrial remodeling
- Endometrial angiogenesis
- Inhibitory effect - GnRH, FSH, LH
- Maintenance of pregnancy



# Inflammatory Response



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# Inflammatory Response



- Inflammation - innate biological response to harmful stimuli → e.g. pathogens, tissue lesions, irritants
- Acute Inflammation
  - Initial response → increased plasma, granulocytes to inflammatory site
  - Vascular component
  - Immune component
- Chronic inflammation
  - Prolonged inflammation → agranulocytes involvement
  - Inflammatory mediators
    - Nitric oxide (NO)
    - Tumor Necrosis Factor-alpha ( $\text{TNF}\alpha$ )

# Progesterone and Inflammation



- P4 → the ‘hormone of pregnancy’
  - Endometrial remodeling & angiogenesis
- P4 → also an immunosteroid
- Not mutually exclusive
  - P4-driven modulation of maternal immune system required for pregnancy to go to term
    - Protection allogeneic conceptus
- P4-R → identified on lymphocytes - pregnant/non-pregnant women, men
  - Peripheral blood monocytes - non-pregnant mice
  - Macrophages - mice

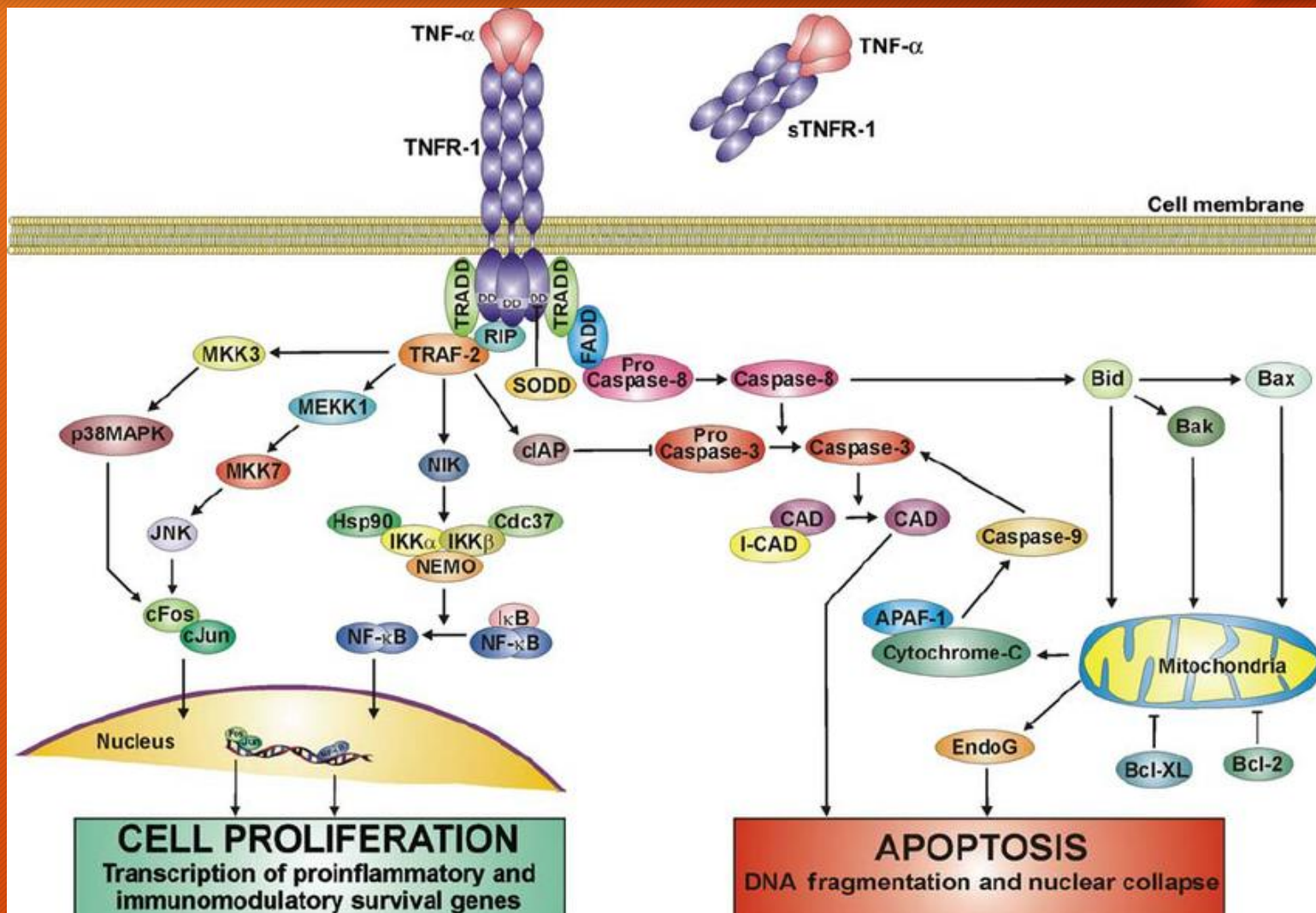


# Progesterone and Inflammation



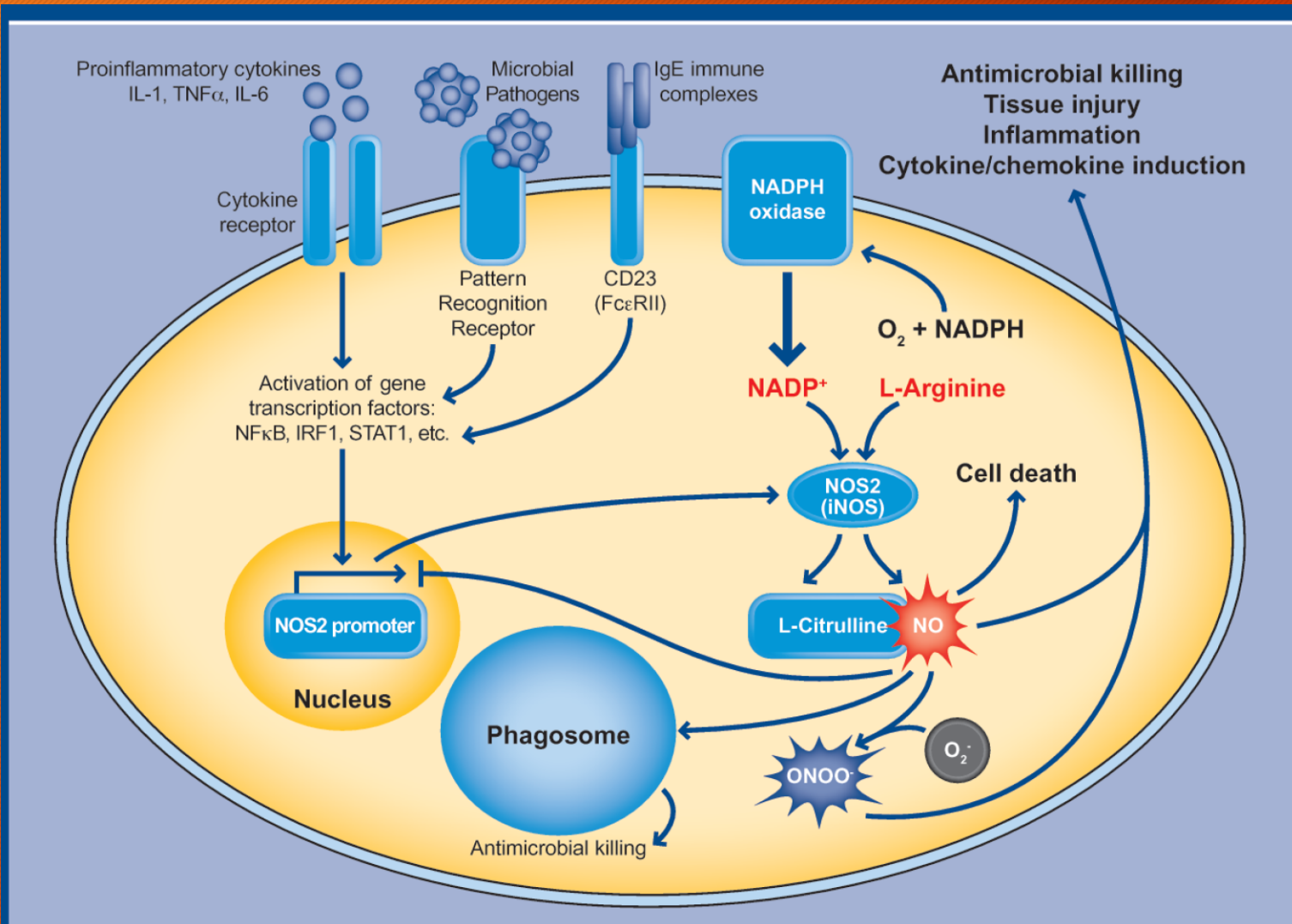
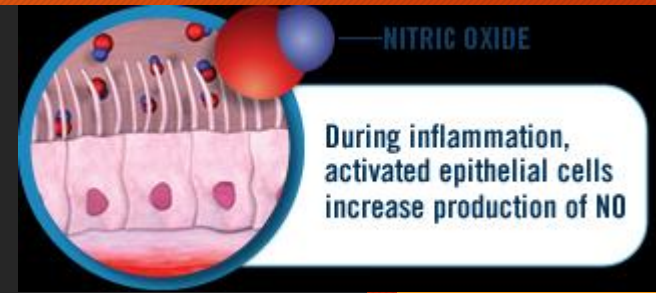
- P4 → neuroprotective effects in acute brain injury
  - Ischemic stroke
  - Subarachnoid hemorrhage
- P4 → promising treatment in traumatic brain injury
  - Phase II & III clinical trials

# Inflammatory Response - $\text{TNF}\alpha$



# Inflammatory Response

## - NO





# Experimental Objectives



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# Experimental Objectives



Ascertain presence of progesterone receptor

- Saturation binding

Functional Assays

- Cell stimulation - LPS
- Inhibition Assays - P4  $\rightarrow$  NO, TNF
- Competition Assays - RU486

# Experimental Objectives



Determine the presence of the progesterone receptor on multiple cell types

Determine saturation point/specificity of binding

## Assays

- Saturation binding - Flow cytometry
- Functional assays (LPS, PGN stimulation: NO,  $\text{TNF}\alpha$ )
- Competition binding - RU486



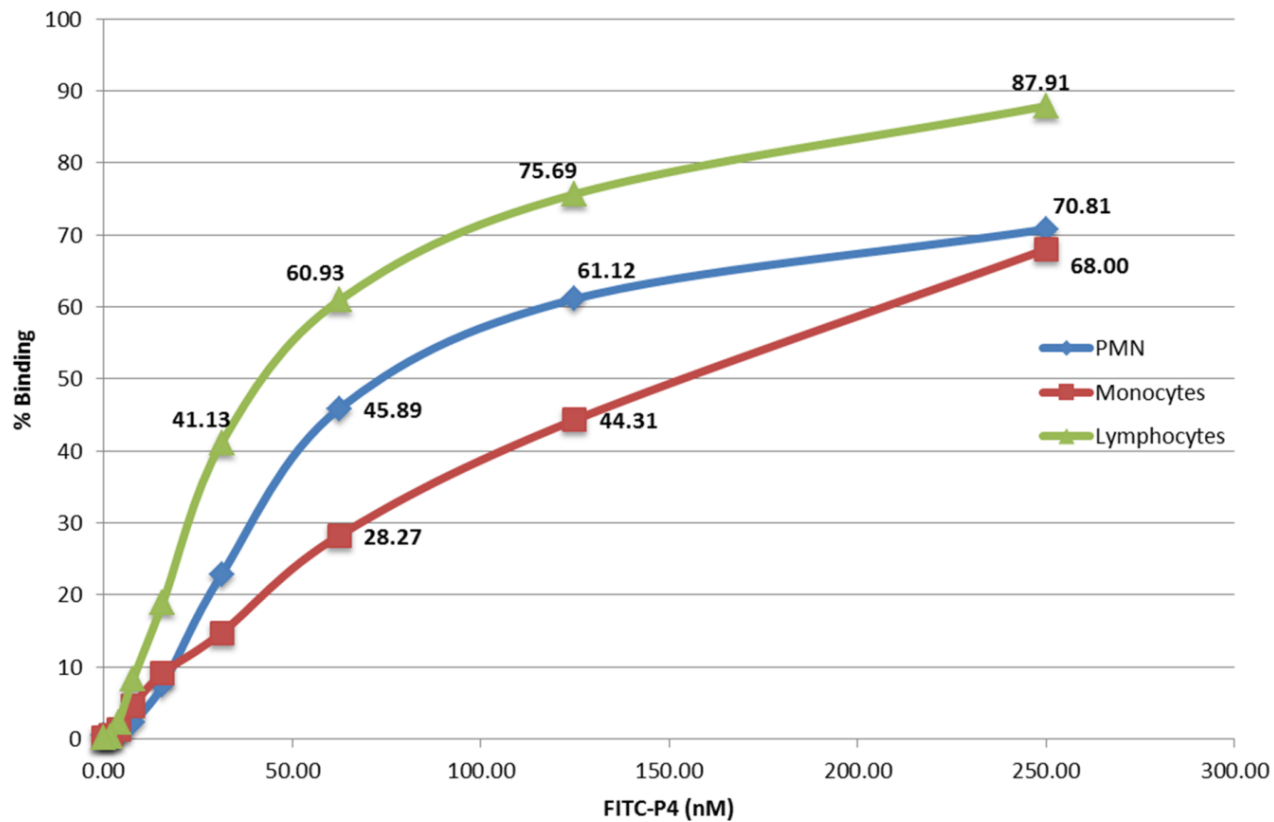
# Results

- Saturation Binding
- Nitric oxide assays
- TNF Assays



# Saturation Binding

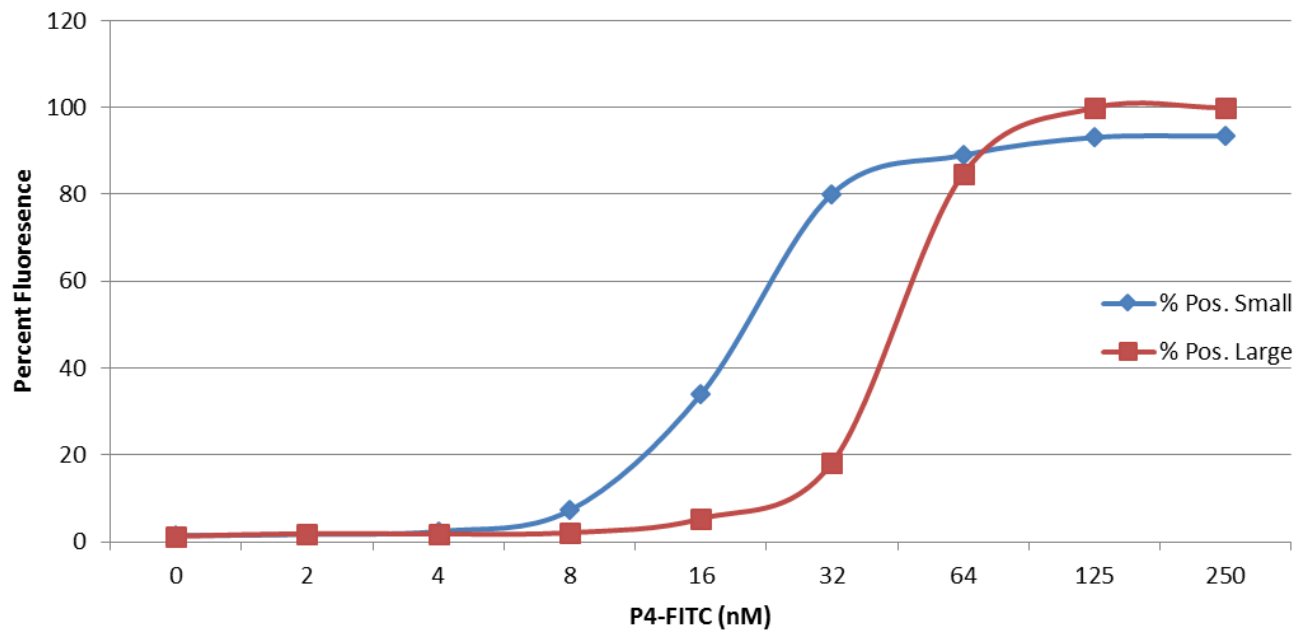
Comparison of FITC-P4 Binding to Bovine Mononuclear Cell Populations



# P4 Saturation Binding



P4-FITC Binding - RAW 264.7

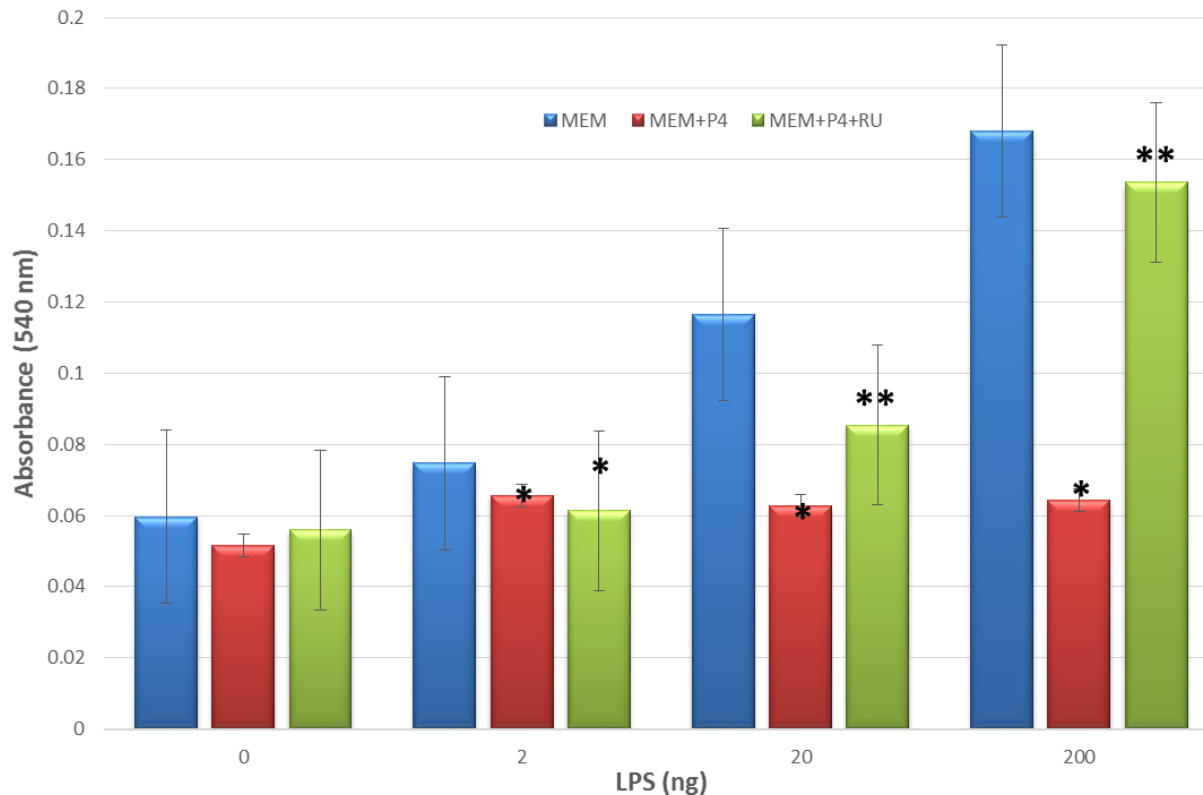




# P4 Inhibition of Nitric Oxide

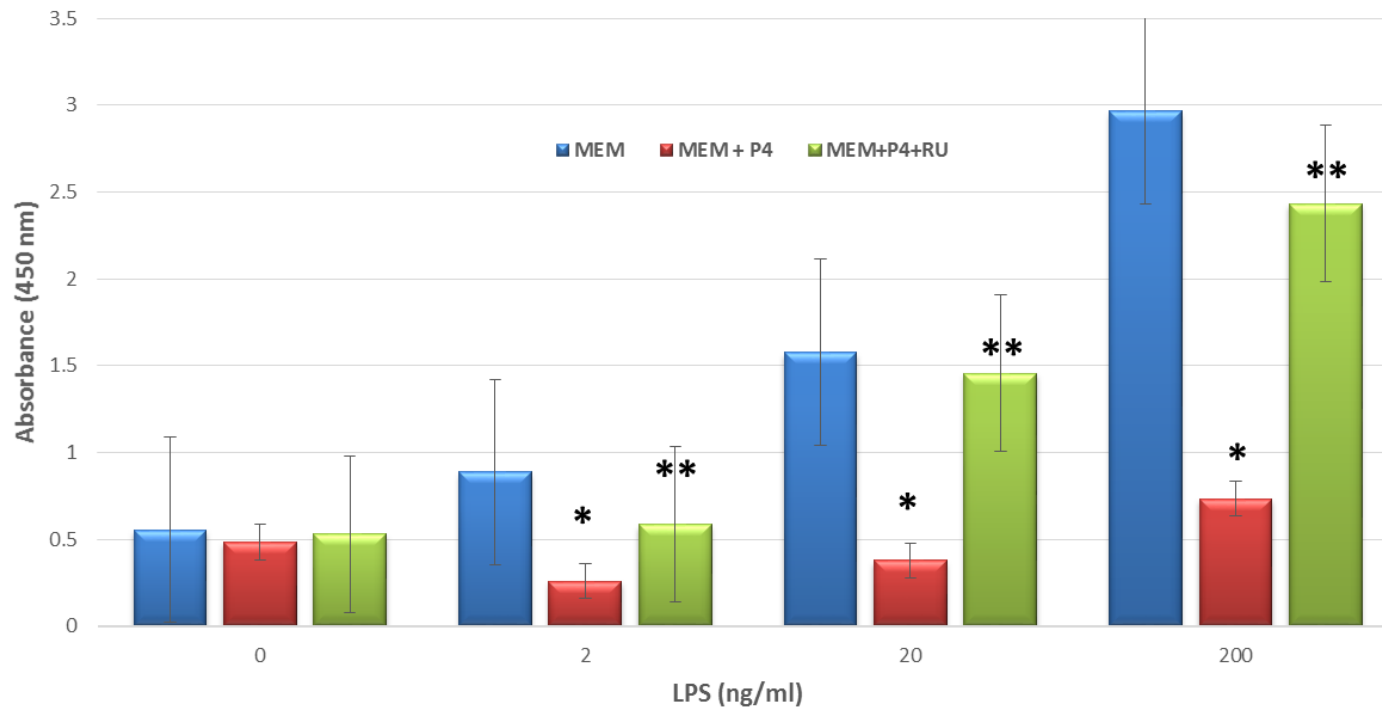


Anti-Inflammatory Properties of Progesterone: Effects of Endotoxin Challenge on Nitric Oxide Production in RAW 246.7 Cells



# P4 Inhibition of $\text{TNF}\alpha$

Antiinflammatory Properties of Progesterone: Effects of Endotoxin Challenge on  $\text{TNF}\alpha$  Production from RAW 246.7 Cells



# Future Objectives



# Looking Ahead



- Effect of LPS/PGN in competition assays
  - RU486
- Effect of P4 on  $\text{TNF}\alpha$  assays
  - Competition
- Intracellular  $\text{Ca}^{2+}$  signaling
- Translation of assays to capacitation
- Sequence receptor?



# Thank You!



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# UNUSED SLIDES



# Capacitation



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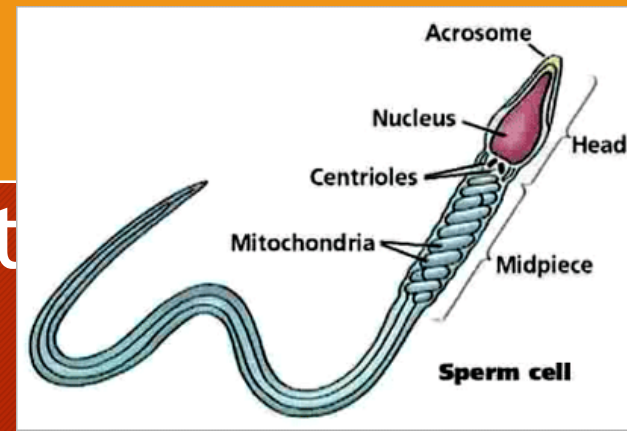
# Capacitation

Biochemical change in sperm resulting in fertilizing capacity

- Phase I: Male reproductive tract
- Phase II: Female reproductive tract

Culminates in the acrosome reaction

- $\text{Ca}^{2+}$ -mediated event





# Capacitation

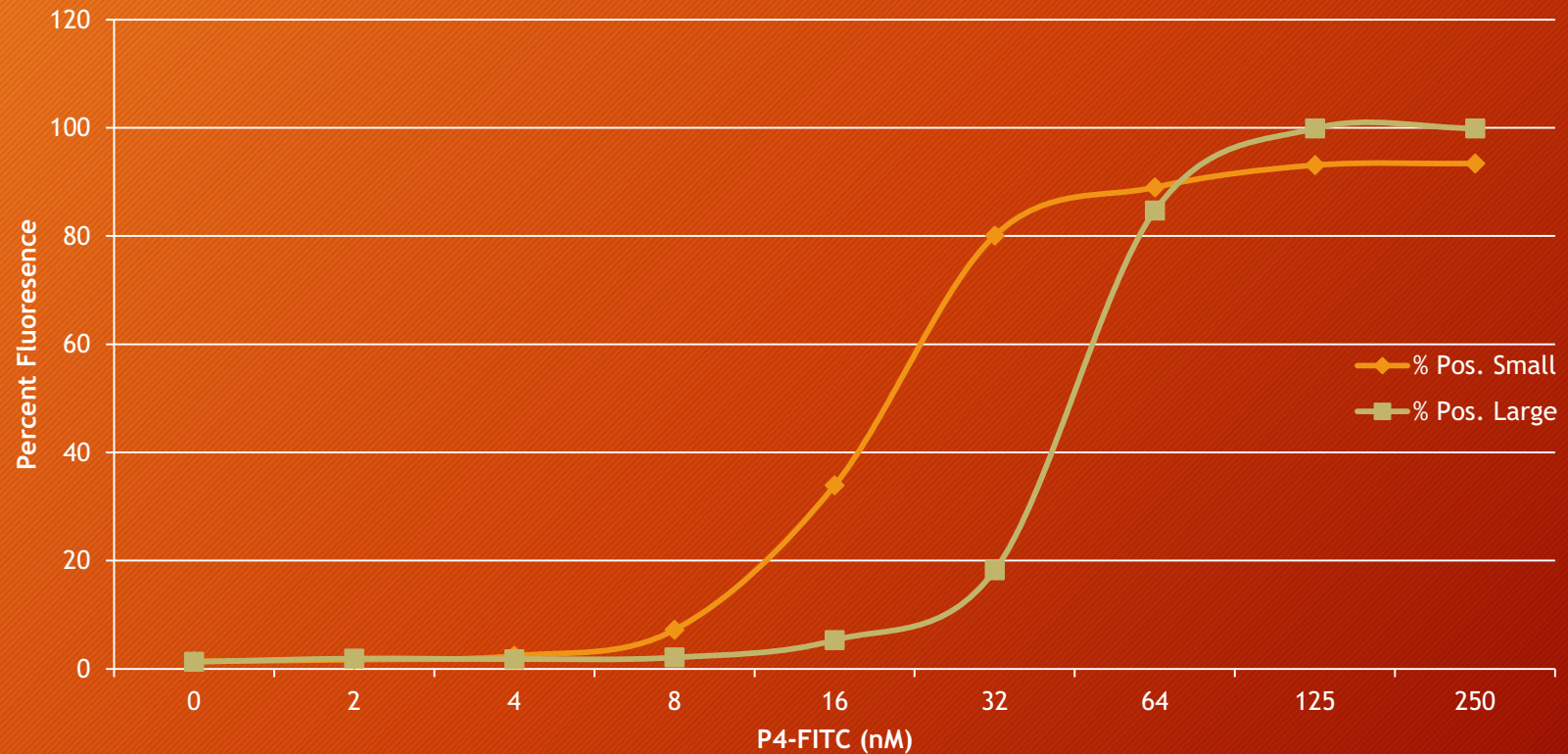
As sperm migrate through female tract, significant increase in intracellular  $\text{Ca}^{2+}$  observed

- Possible mechanism: progesterone signaling

**Hypothesis:** incubation of spermatozoa with progesterone will result in significant increase in intracellular calcium

# Saturation Binding

## P4-FITC Saturation Binding - RAW 264.7



# Nitric Oxide Assays

## Nitric Oxide Assay - LPS Stimulation



LPS stimulation revealing maximal response of Nitric oxide to 2000 ng LPS. A mean 60% dose-response inhibition of nitric oxide production observed in cells incubated in saturation concentration of progesterone. All data point represent experiments in triplicate.