



Does Pregnancy Lead to the Development of Anti-Cyclic Citrullinated Peptide (CCP) Antibodies?

Rosalyn Savoie¹, Ashley Visser², and M. Kristen Demoruelle²

1. University of Colorado School of Medicine, Anschutz Medical Campus, Aurora, CO; 2. University of Colorado Denver, Division of Rheumatology, Aurora, CO

INTRODUCTION

Before the onset of rheumatoid arthritis (RA), there is a **pre-clinical period** where patient genetics and environmental factors lead to the **development of RA-related autoantibodies years before the onset of inflammatory arthritis**.

The current understanding of RA development fails to account for sex differences in RA:

- 3-fold risk for women to develop RA compared to men
- **6-fold increased risk of developing RA postpartum**¹

These sex differences in RA development could potentially be due to changes during pregnancy that lead to the **development of anti-citrullinated peptide antigens (ACPAs)**, which are antibodies that are associated with the development of RA and are highly specific for RA.²

One potential link between RA and pregnancy could be via neutrophil extracellular traps (NETs). NETs are a form of neutrophil cell death where the neutrophil releases its DNA in order to trap pathogens, and the formation of NETs is termed NETosis. It is known that increased levels of NETosis occurs with:

- **Pregnancy:** Increasing gestational age leads to **increased NETosis**.³
- **RA development:** NETosis can externalize citrullinated proteins that can be the antigenic **target of ACPA**.⁴ NETosis is also elevated in RA.

Based on these factors, **we hypothesized that changes in pregnancy which lead to increased NETosis can externalize citrullinated proteins that trigger the generation of ACPA**. Subsequently, increased ACPA generation in pregnant women could increase the risk of developing RA postpartum.

To test this hypothesis, we evaluated the prevalence of ACPA in the serum of pregnant and non-pregnant women using cyclic citrullinated peptide (CCP) assays.

METHODS

Study subjects: From the Baby Blanket study biorepository:

- 340 **pregnant** women without RA in their 3rd trimester of pregnancy

From the Studies of the Etiology of RA (SERA) cohort:

- 142 **non-pregnant**, pre-menopausal women without RA

ACPA testing: Serum samples were tested for ACPA using CCP3 (IgG) and CCP3.1 (IgG/IgA) ELISA assays. CCP positivity was established using the manufacturer's recommended cut-off level for positivity. Questionnaires were administered to assess women's health and smoking history.

Analysis: Fisher's exact test and chi-squared tests were used to compare anti-CCP3 and anti-CCP3.1 positivity between groups.

RESULTS

Figure 1. Hypothesis

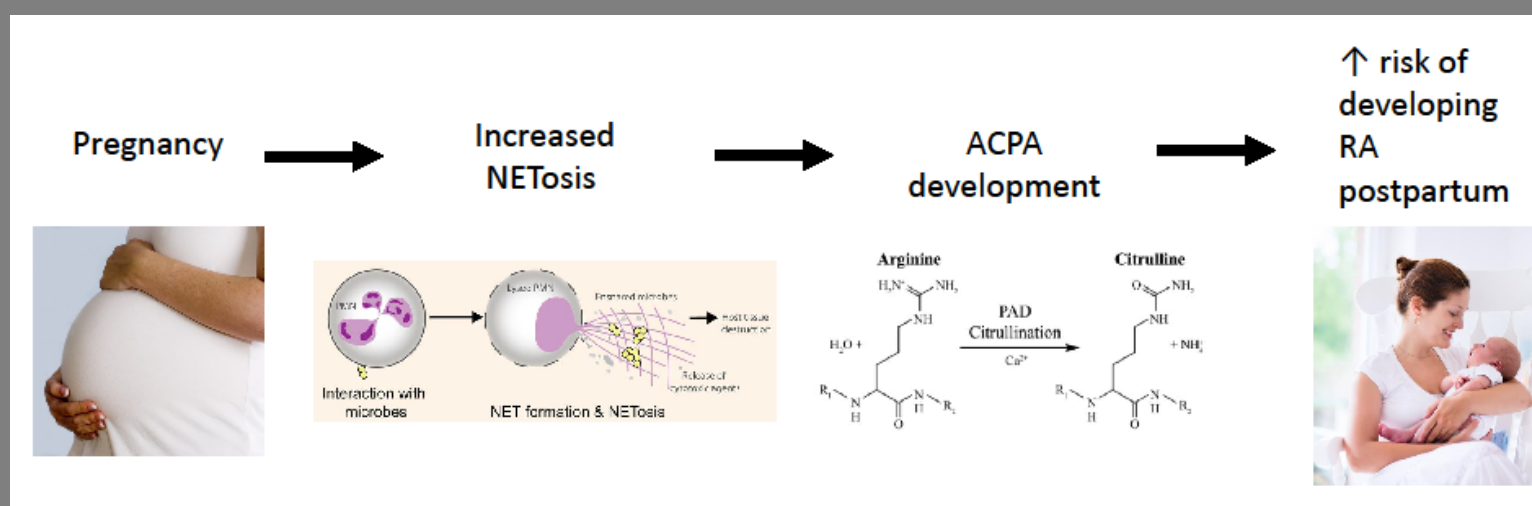


Figure 2. Phases of RA Development

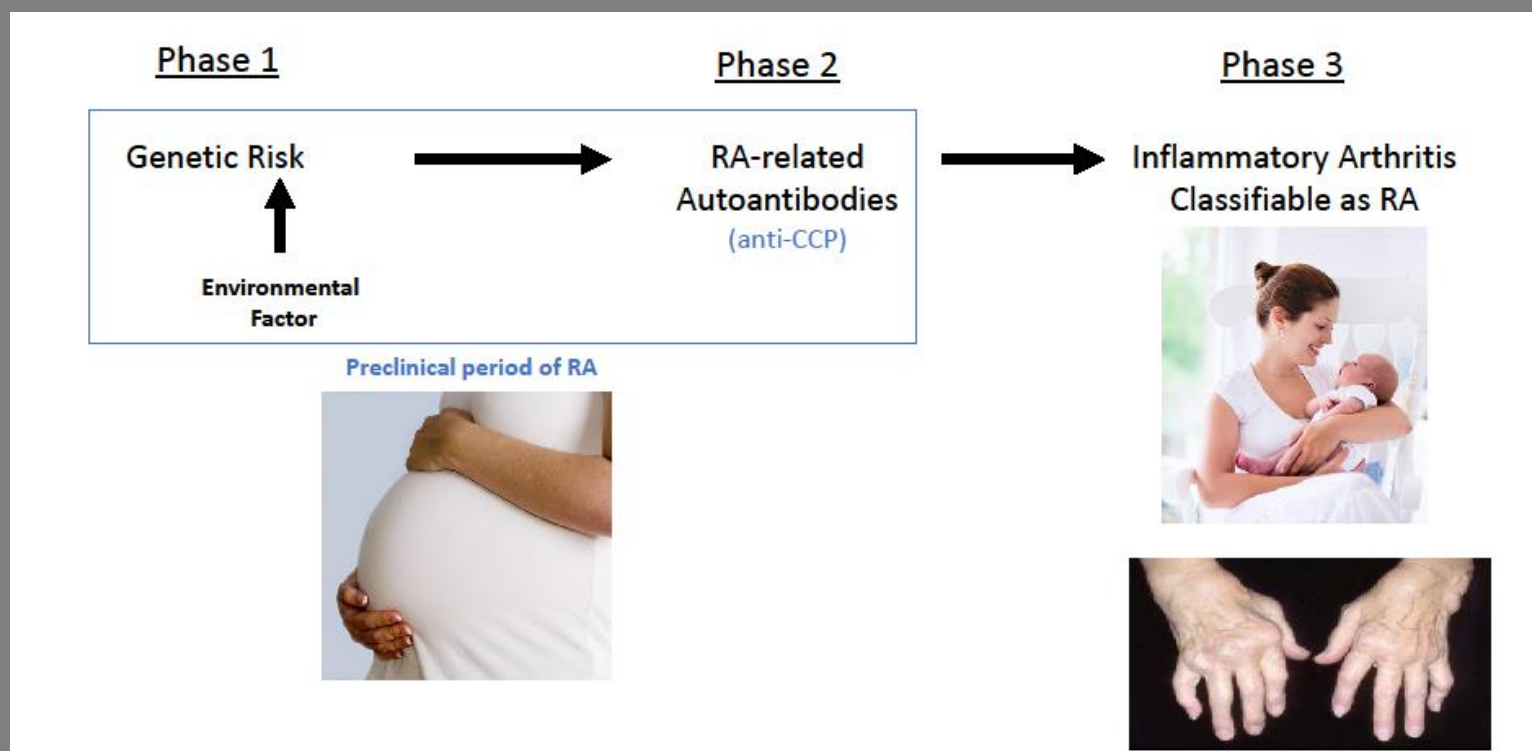


Table 1. Subject Characteristics

	Pregnant (n = 340)	Non-pregnant (n = 142)	P-value
Ever pregnant	100%	31%	<0.01
Average age (SD)	30 (5)	31 (8)	0.02
Ever smoker	30%	15%	<0.01
Family history of RA	0%	5%	<0.01

Table 2. Anti-CCP Positivity

	Pregnant (n = 340)	Non-pregnant (n = 142)	P-value
Anti-CCP3 Positive	2.1%	1.2%	0.43
Anti-CCP3.1 Positive	1.4%	1.5%	1.00

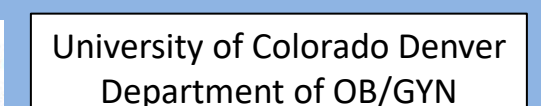
Major Findings

- There was no difference in serum anti-CCP positivity between pregnant and non-pregnant women.
- Similar results were demonstrated when comparing pregnant women vs. never-pregnant women ($p = 0.70$ and $p = 1.0$).
- Within pregnant women, there were no differences in serum anti-CCP positivity related to:
 - Age
 - History of smoking
 - History of sexually transmitted disease

CONCLUSION

- Pregnancy is not associated with systemic elevations of anti-CCP antibodies.
- However, none of the pregnant women had a family history of RA, so it is unclear if genetics affects the development of ACPA in pregnant women.
- Future studies are needed to understand the additional factors related to pregnancy that could lead to the development of RA.
 - Does genetic risk or family history of RA increase anti-CCP generation during pregnancy?
 - Could anti-CCP antibody generation occur at a local mucosal level in the female genital tract during pregnancy before becoming systemic during the postpartum period?
 - What other factors during pregnancy lead to the development of RA postpartum?

These studies were supported by the following:



References: 1) Scott, D., The Lancet, 2010 2) Kuhn, K., The Journal of Clinical Investigation, 2006 3) Giaglis, S., Frontiers in Immunology, 2016 4) Khandpur, R, Science Translational Medicine, 2013