ImmunoTools *special* Award 2026

Rosanna Ramhorst, PhD, Placenta Lab, Jena University Hospital, Jena, GERMANY

Udo Markert, MD, Professor, Placenta Lab, Jena University Hospital, Jena, GERMANY

Regulation of senescence in endometrial aged: impact in endometrial receptivity

TRANSLATIONAL IMPACT OF THE PROPOSAL

Currently, more and more women around the world are deferring motherhood, and this phenomenon is also observed in Germany and Argentina. A series of variables could explain this trend: the professional, personal, and/or financial maturity of the woman, which usually occurs during her fertile period, as well as the desire to form a couple and a conjugal family within the framework of which to have children. In women, advanced age decreases the ovarian reserve and impacts the reprogramming of endometrial cells (*Zegers-Hochschild 2021, Glujovsky 2020*). This is why it is key to address the mechanisms that participate in endometrial aging in relation to embryo implantation and achieving a full-term pregnancy. This information can provide recommendations for future research focusing on molecular mechanisms of age-related cellular senescence, cellular composition, and transcriptomic changes in relation to endometrial aging. Additionally, further prospective research is needed to explore newly emerging therapeutic options, such as senolytic agents that can target endometrial aging without negatively affecting endometrial functioning; therefore, further research is needed to

understand the unique mechanisms involved that affect receptivity and pregnancy outcomes.

In the present proposal, we seek to generate a collaborative network between researchers from Prof. Dr. Udo Markert's Lab and Prof. Dr. Ramhorst's Lab in order to address "delayed motherhood and the impact of endometrial senescence." The collaboration between both groups has been ongoing since our joint participation in conferences and scientific committees of Reproductive Immunology Societies. We have had the opportunity to discuss our results during his several scientific visits to Buenos Aires and at different regional SLIMP symposia (in Argentina, Chile, and Brazil). Certainly, this collaboration was consolidated within the framework of a DAAD grant, and I am (Rosanna) currently working at Prof. Udo Markert's lab until 2026.

BACKGROUND AND GENERAL CONSIDERATIONS OF THE PROPOSAL

Until recently, the study of age-related decline in fertility has focused primarily on the ovary, through which fertility declines with advancing age; however, little is known about the impact of age on endometrial function. The endometrium represents a unique tissue due to its ability for cyclic regeneration, and it comprises many cell types, including epithelial, stromal, vascular, immune, and stem cells, which function in a coordinated manner (*Chemerinski 2024*).

In this sense, endometrial receptivity implies the differentiation of endometrial stromal cells, known as the "decidualization program." This is a multistep process of differentiation leading to a balance between two subpopulations of cells: mature and senescent decidual cells (*Brighton 2017, Altmae 2012*). We propose that in the aged endometrium, senescent decidual cells increase due to a less effective clearance of senescent cells by immune cells. Therefore, there will be an increase in SASP production (senescence-associated secretory phenotype: a battery of pro-inflammatory cytokines and chemokines). The SASP secreted by senescent cells may induce paracrine senescence in neighboring cells and may disturb endometrial integrity.

In other words, in a young endometrium, two subpopulations—mature and senescent decidual cells—coexist, and the clearance of senescent decidual cells by immune cells controls this balance, contributing to endometrial receptivity. We propose that in an aged endometrium, the removal of senescent cells will be affected, increasing SASP mediators, which may transmit senescence to neighboring cells, thus reducing endometrial receptivity.

Particularly, during my stay at the Placenta Lab, we will focus on the clearance of senescent endometrial cells by immune cells, particularly $CD8^+$ and NK cells, associated with endometrial aging.

METHODOLOGY

To address the impact of endometrial senescence on delayed motherhood, our research focuses on how immune cell populations regulate the removal of senescent cells. We will use **CD3 APC**, **TCRαβ PE**, and **CD8a APC** from *ImmunoTools* to characterize the cytotoxic T cell compartment.

For the study of NK cells, **CD56 APC** and **CD16 PE** will allow us to distinguish between decidual and peripheral subsets, while **CD314 (NKG2D) APC** will be used to assess the recognition of senescent-related ligands. The cytotoxic functionality of these cells will be directly quantified through the expression of **Granzyme FITC**.

To investigate the potential role of immunosuppressive environments in the aged endometrium, we will apply **CD33 APC**, **CD14 FITC**, **CD14 PE**, and **CD11c PE** to identify myeloid-derived suppressor cells (MDSCs) and dendritic cells that may interfere with receptivity. Meanwhile, the imbalance between mature and senescent subpopulations — a hallmark of reduced receptivity — will be evaluated using **Annexin V FITC** and **Annexin V APC** combined with other viability markers to detect apoptosis and cell death following immune interaction.

Furthermore, we will analyze the Senescence-Associated Secretory Phenotype (SASP) and its impact on endometrial integrity. Particularly, the production of inflammatory cytokines which amplifies senescence such as TNF-**α** and IFN-**γ**. Specifically, **TNFα FITC** will be used to monitor pro-inflammatory and angiogenic signaling, while the **anti-mouse PE secondary antibody** will facilitate the detection of immunomodulatory factors. Finally, a **human IFN-γ ELISA** will be employed to further characterize the secretory phenotype associated with cytotoxicity.

This research is particularly relevant in the context of the global trend toward delayed motherhood. By integrating phenotypic characterization of cytotoxic and immunosuppressive populations, this project seeks to elucidate how aging reshapes immune homeostasis in the female reproductive tract. Ultimately, these findings aim to bridge the gap between basic reproductive immunology and clinical practice, offering a foundation for novel therapeutic strategies to improve pregnancy outcomes in women of advanced maternal age.

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