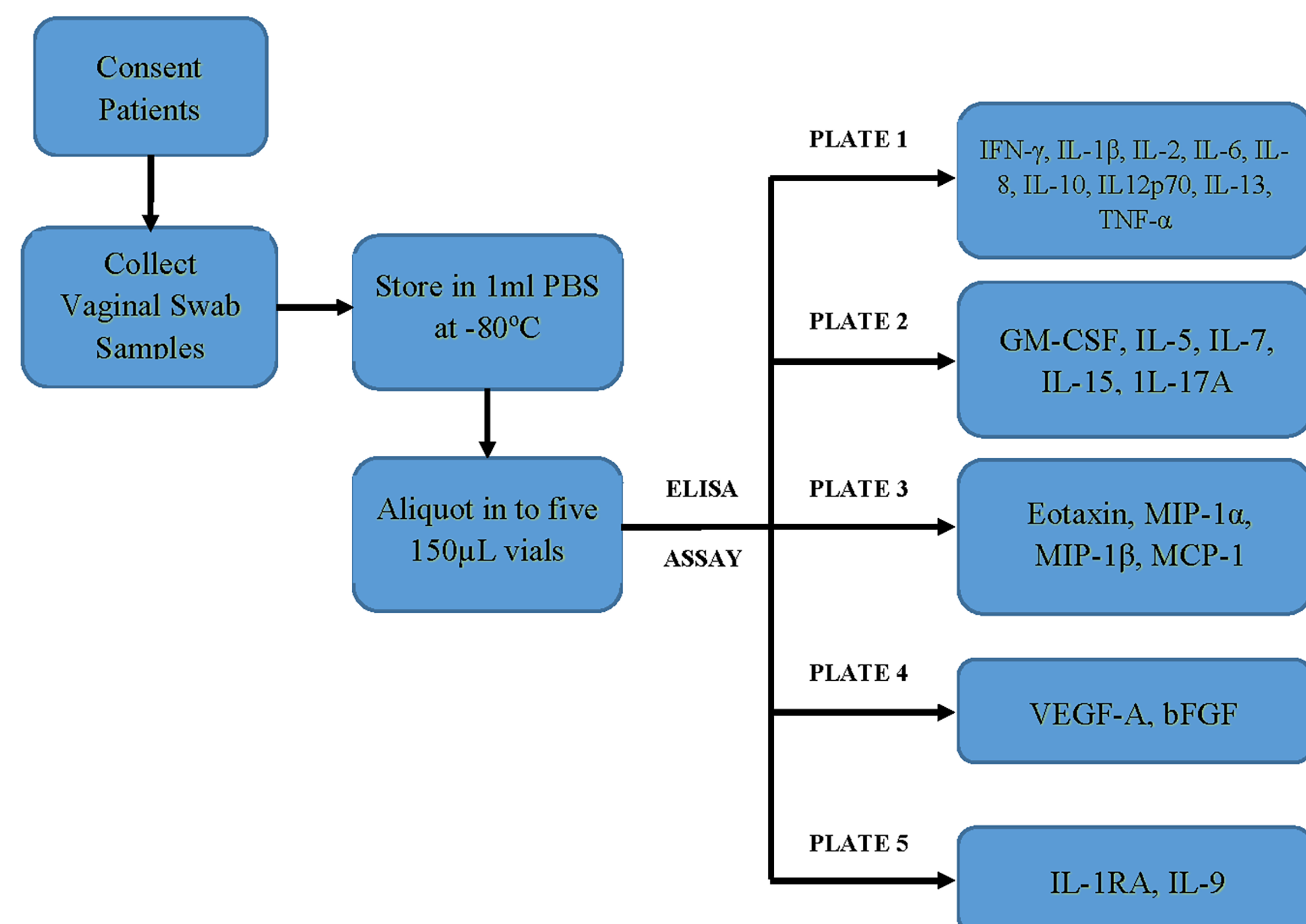


INTRODUCTION

- Immunologically, the fetus is observed as an allogenic graft during pregnancy that requires careful maintenance of the maternal immune system¹. Temporary immune suppression is an essential component in pregnancy that protects the fetus from the maternal immune system².
- Increases in pro-inflammatory signaling during pregnancy is associated with preterm birth, pre-eclampsia, and more seriously, miscarriage³. It is known that in women who have uncomplicated pregnancies, there is an overall decrease in pro-inflammatory cytokines and increased counter-regulatory cytokines⁴.
- While most of the literature focuses on studying the reproductive immune system during pregnancy and the potential pathology associated with disturbed immunity, there is not much to be said regarding particular immunological differences in woman who have never had a pregnancy and women who have had a successful pregnancy.
- The objective of this research is to identify whether there is a measurable difference between the cytokines expressed in women who have had a successful pregnancy and not currently pregnant and women who have never become pregnant.

MATERIALS AND METHODS

- The study was approved by the IRB from the Texas Tech University Health Sciences Center, TX.
- Vaginal swab samples were obtained from a total of 18 patients, 11 from the parous group, and 7 from the nulliparous group.
- The swab samples were then placed in vials with 1mL of Phosphate saline buffer (PBS) and stored in -80°C.
- The samples were then placed in to five 150µL aliquots for five ELISA assays, each plate measuring various cytokines. Refer below the schema.
- Statistical analysis was performed using R Studio program with a significance level of $\alpha = 0.05$.



RESULTS

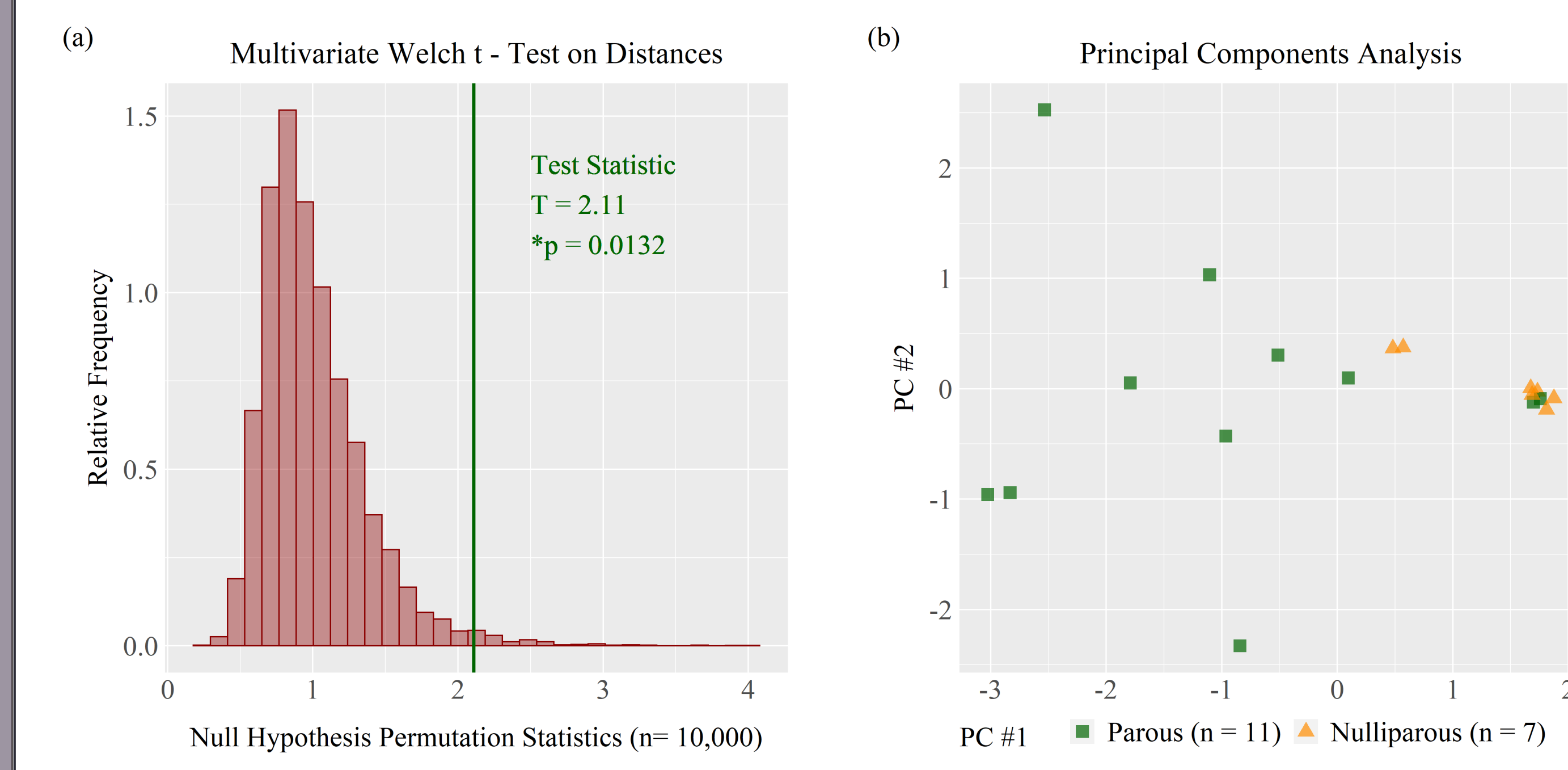


Figure 1: Multivariate Welch t-Test on Distances of null hypothesis (a) and Principle Coordinate Analysis (PCA) plot (b). Cytokine levels are significantly different between parous and nulliparous group ($*p=0.0132$). PCA was performed to confirm shifts in cytokine levels between two groups.

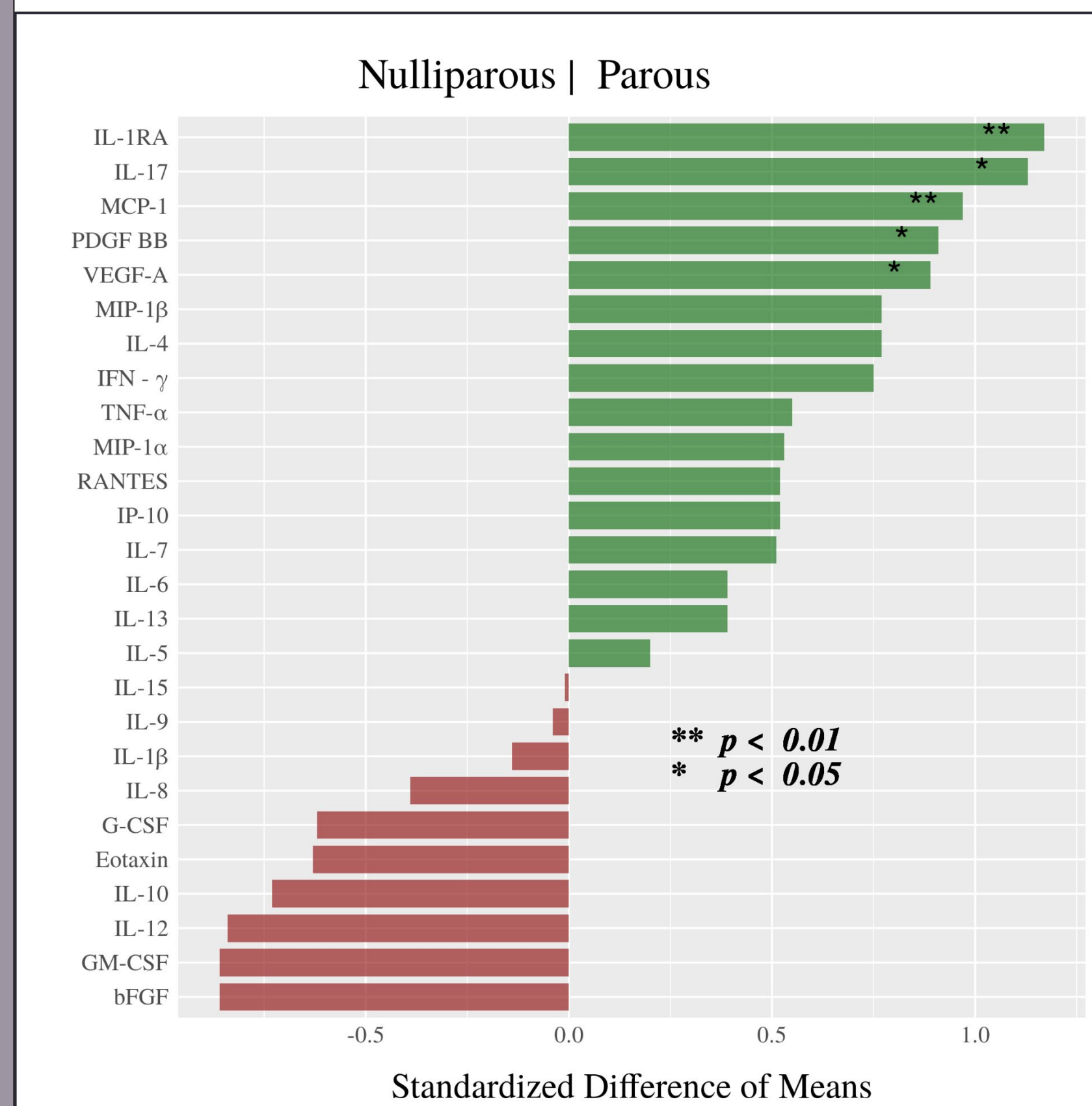
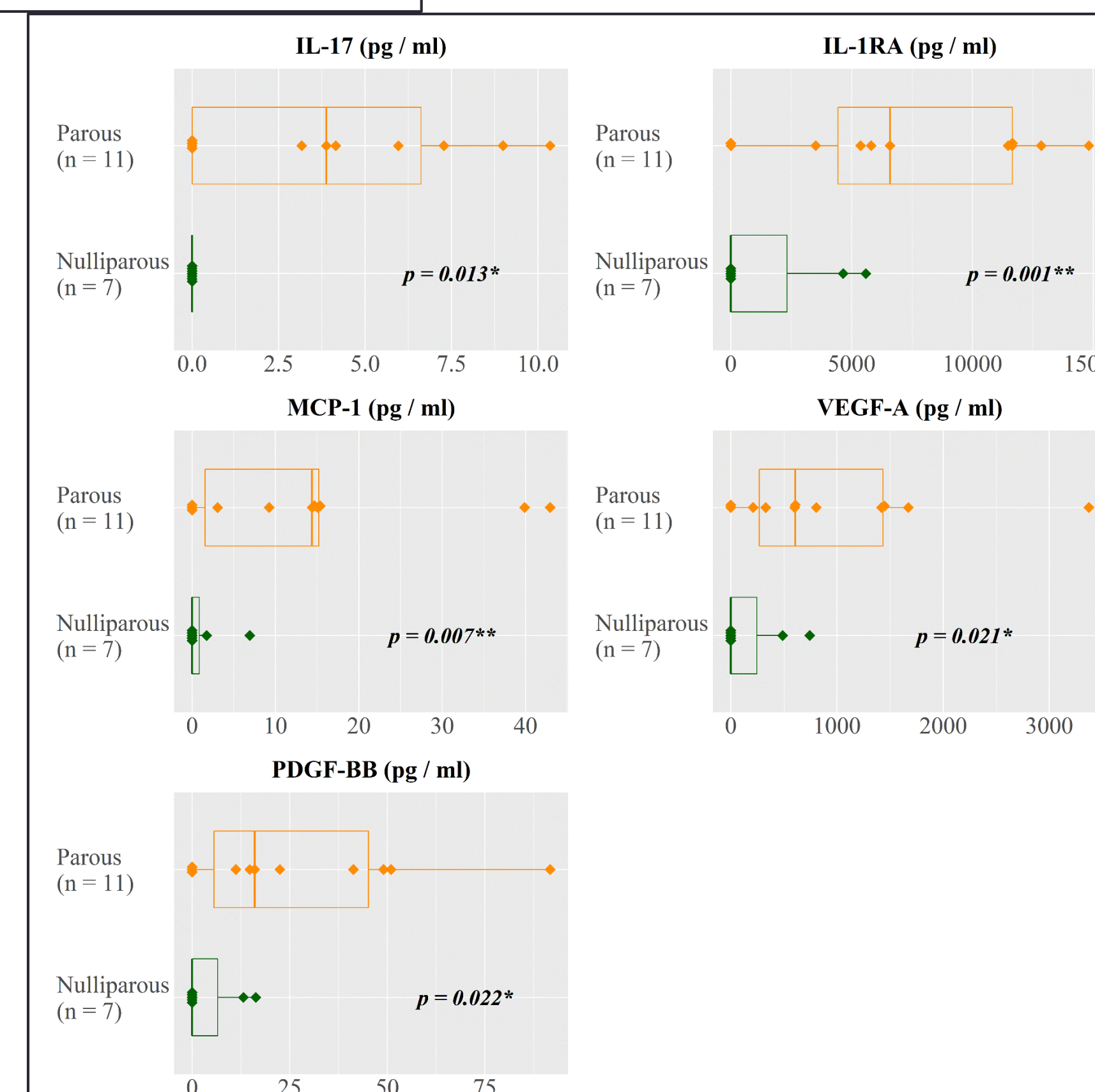


Figure 2: Standardized difference of means for cytokine concentrations. Measured differences between the parous and nulliparous group. ($*p<0.05$, $**p<0.01$, $***p<0.001$)

Figure 3: Statistically significant differences in cytokine distributions. IL-17, IL-1RA, MCP-1, VEGF-A, and PDGF-BB are expressed significantly higher in parous group than the nulliparous group. ($*p<0.05$, $**p<0.01$, $***p<0.001$).



DISCUSSION AND CONCLUSION

- In our study, five cytokines were observed to be significantly elevated in the parous group: IL-17, IL-1RA, MCP-1, VEGF-A, and PDGF-BB.
- IL-17 is a major pro-inflammatory marker which plays a critical role in angiogenesis and immune regulation.
 - Can be present in the human placenta
 - However, excessive expression of IL-17 has been associated with complications such as fetal growth restriction and preeclampsia⁵.
- IL-1RA, a natural inhibitor of IL-1β, was found to be significantly decreased in the parous group when compared to the nulliparous group
 - Can be present in amniotic fluid and cord blood; also circulates systemically higher concentrations in pregnant women⁶.
 - Can provide protective benefits to mother and developing fetus^{6,7}.
- MCP-1 also known as chemokine ligand-2 (CCL2), was significantly higher in concentration in the parous group
 - Can be present in the decidua, chorion and amniotic fluid
 - Regulates migration and infiltration of monocytes/macrophages^{8,9}.
 - Unregulated increases in MCP-1 secretion during pregnancy may contribute to preeclampsia.
- VEGF-A and PDGF-BB expression was significantly elevated in the parous group when compared to the nulliparous group
 - VEGF-A has been found to be irreversibly bound in the amniotic fluid during pregnancy¹⁰.
 - Both acts on endothelial cells inducing growth and repair of tissues which ensures normal fetal growth and healthy parturition^{10,11}.
- In conclusion, our study determined that there is a step change in cytokine expression when comparing nulliparous and parous groups.
 - More research is required to clarify the functionality of measuring cytokine levels as an indicator for pregnancy health and fertility.

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