

学会名

IFFS 2025 と第 70 回の生殖医学会

Poster Presentation(ポスター発表)

都市, 国名, 日程(西暦.月.日)

東京国際フォーラム, 東京, Japan. (2025 April 26-29)

題名

**The Follicular Immune Landscape May Predict Embryo Developmental Competence**

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**Introduction**

The ovarian follicle is a complex and dynamic microenvironment that plays a crucial role in oocyte development and fertility. This structure comprises granulosa cells, cumulus cells, and diverse immune cells that contributing to oocyte meiotic resumption, cellular chemotaxis, and extracellular matrix remodeling during ovulation. However, the relationships between immune cell dynamics, patient characteristics, and their roles in fertilization and embryo development remain unclear. This study investigates the relationship between follicular fluid (FF) immune cell dynamics, patient characteristics, and embryo development.

**Method**

Following the Institutional Review Board approval and informed consent from the participating patients, the FF samples were carefully collected from the first punctured follicle with a diameter of 18-22 mm and containing cumulus-oocyte complexes, from a total of 54 patients. The oocytes were individually cultured following intracytoplasmic sperm injection procedures and the embryos were assessed for morphological characteristics and evaluated using an AI-based time-lapse scoring (iDAScore) system. The FF cells were stained with different specific fluorescent antibody markers (CD45, CD68, CD80, CD11c, CD56, CD3, CD4, CD25), mainly to identify macrophages, dendritic cell, natural killer (NK) cell, natural killer T (NKT) cell, T cell and regulatory T (Treg) cells. The proportion of different immune cells were analyzed by flow cytometer and compared with the patient's backgrounds, oocytes conditions, fertilization and embryo conditions.

**Results**

The percentage of CD45+ immune cells in FF negatively correlated with patient age. While not statistically significant, BMI trended towards a negative correlation, and anti-Müllerian hormone levels trended towards a positive correlation with FF immune cell percentage. Interestingly, the percentage of immune cells in the FF was significantly and positively correlated with the iDAScore values on day 5, especially for morphologically high-quality embryos. Further, NKT cell percentage negatively correlated with day 3 iDAScore values, while Treg percentage showed a positive correlation with day 3 iDAScore values and was significantly higher in follicles yielding transferable embryos.

**Conclusion**

Our findings suggest that FF immune cells and their secreted factors play a crucial, yet unexplored, role in regulating oocyte maturation, fertilization, and embryo development. Further research is warranted to elucidate the specific mechanisms by which these cells influence reproductive outcomes.