

First steps to define the granulosa cell isolation protocol from human follicular fluid

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Introduction

Infertility affects about 48.5 million couples globally, as reported by WHO [1]. In some women, the low fertility rate is due to a high number of immature eggs in ovarian follicles, which do not mature sufficiently for fertilization. In vitro maturation (IVM) of eggs is a promising solution. Understanding follicular cells like granulosa, cumulus, and endothelial cells is crucial for improving this process. Granulosa cells play a vital role by providing support, growth signals, and hormonal signals to the egg during its development, especially through follicle-stimulating hormone (FSH) receptors. Activation of FSH receptors on granulosa cells triggers biochemical events necessary for follicle development and estrogen production [2-4].

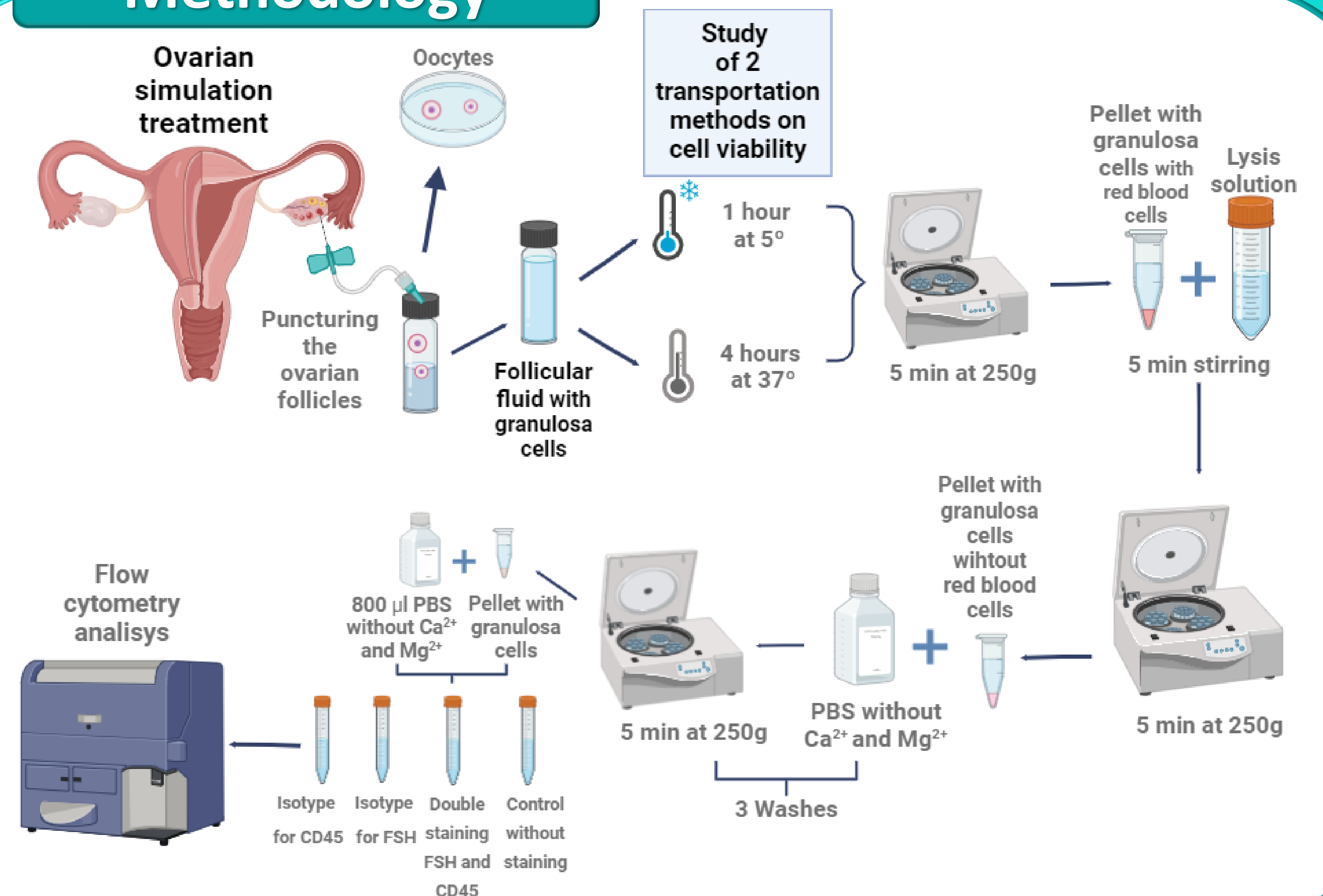
Objectives

Establish an efficient system for isolating granulosa cells in follicular fluid.

Specific objectives:

- Reduce the number of red blood cells present in the follicular fluid.
- Determine the percentage of presumptive granulosa cells (FSH-positive cells) in the follicular fluid.
- Assess the effect of the transport method (4 hours at 37°C vs. 1 hour at 5°C) on cell viability.

Methodology



Results

- Cytometry results show that the 1h transport method at 5°C maintains better viability than the 4h method at 37°C

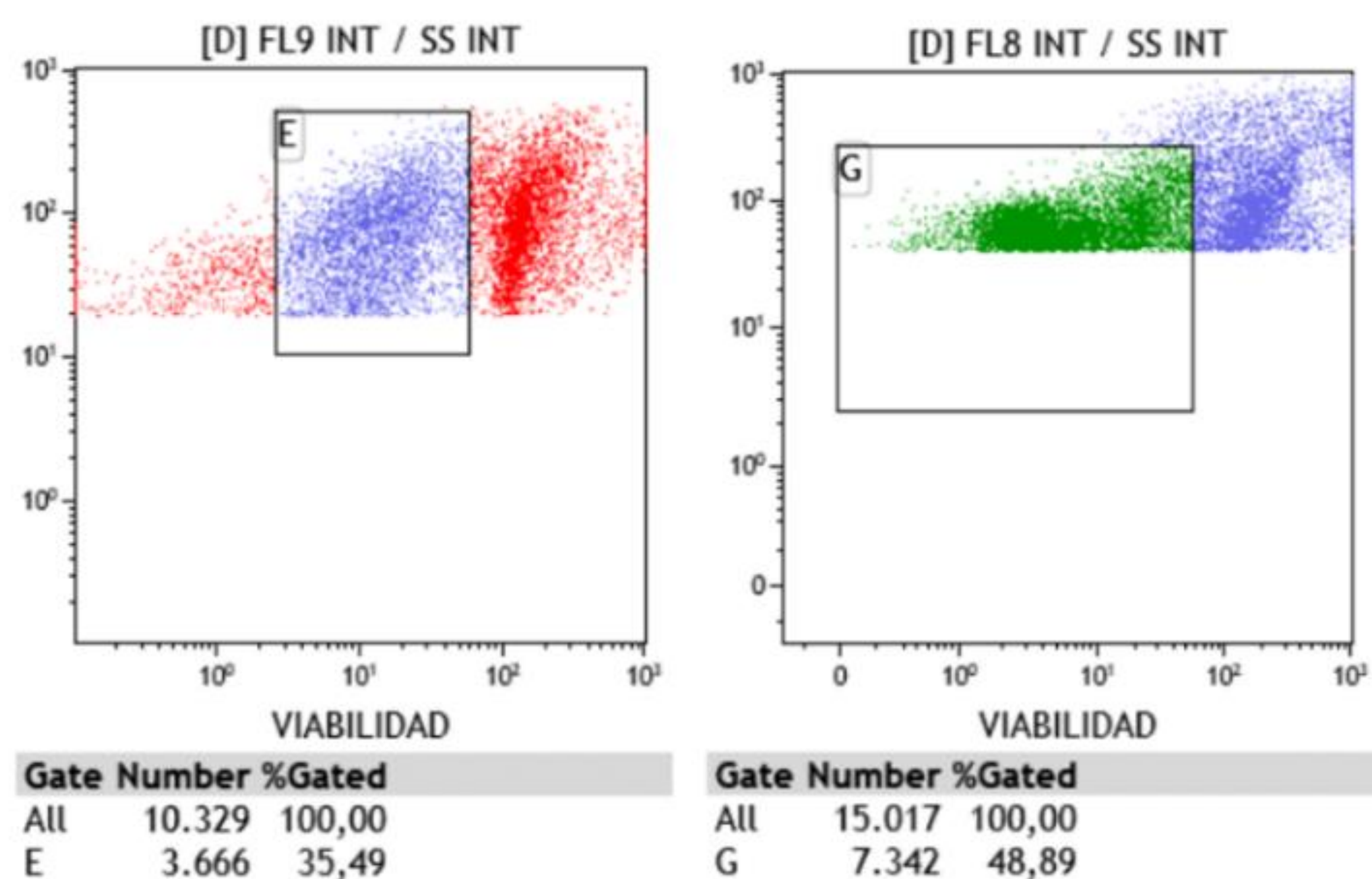


Fig. 1. A: Size/complexity dot plot for viability analysis of the cellular population of interest in the 4-hour sample stored at 37°C. Fig. 1. B: Size/complexity dot plot for viability analysis of the cellular population of interest in the 1-hour sample stored under cold conditions post-collection.

- 77% of the samples do not correspond to cells of the white series (CD45 negative) and do not show FSH receptor (typical of granulosa cells)

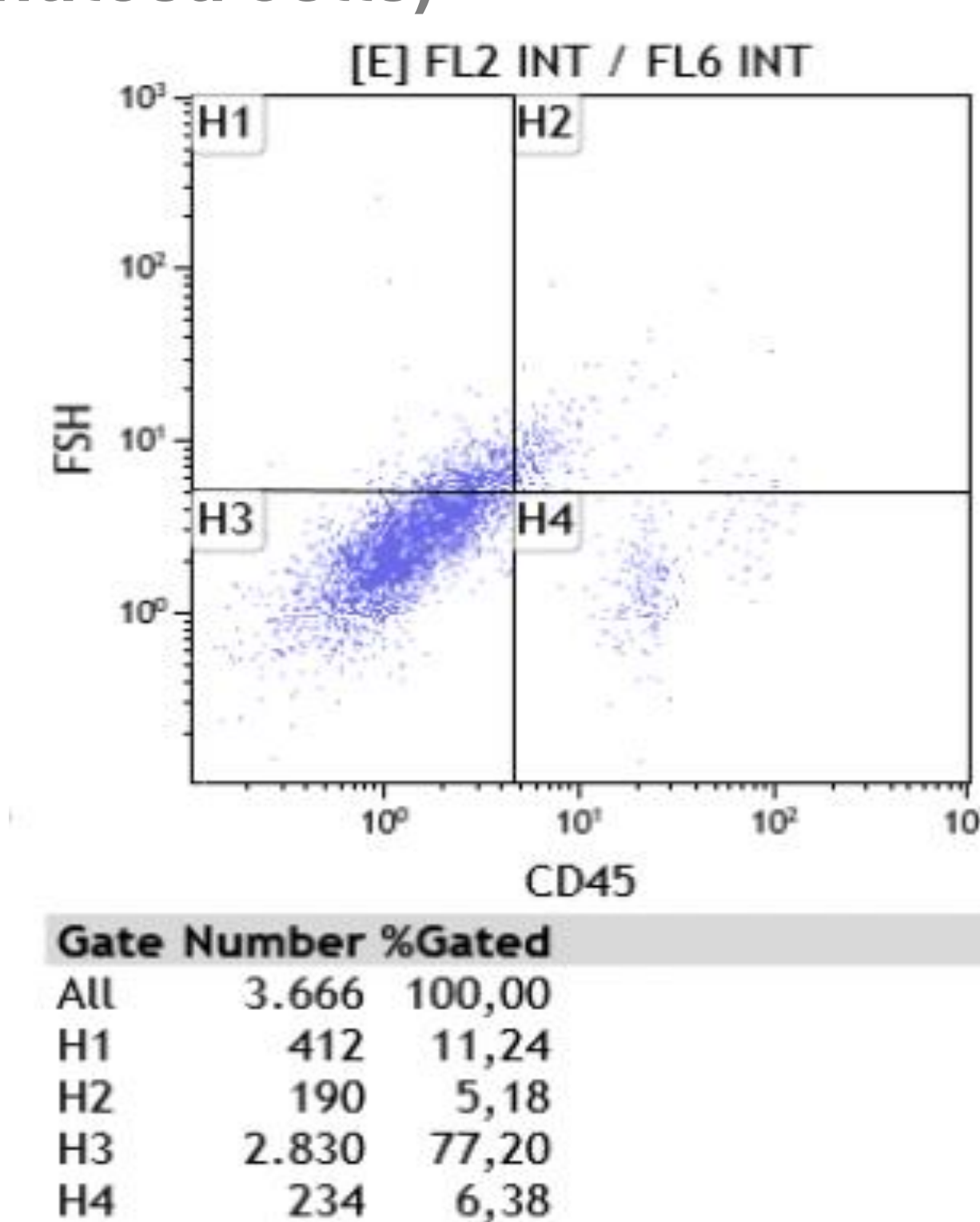


Fig. 2: Graphical representation of the different cellular populations identified for CD45 and FSH receptor antibodies.

Conclusions

- A significant population of FSH receptor-positive cells, characteristic of granulosa cells, was not found; only 11.24% of the total population tested positive.
- The viability achieved with the 1-hour processing method at 5°C post-sample collection is higher compared to samples processed after 4 hours at 37°C

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References

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