Hematological disease represents the most frequent indication for ovarian tissue cryopreservation. However, for patients with acute leukemia, ovarian tissue reimplantation is not safe because of the risk of reintroducing the disease. Grafting of isolated follicles enzymatically purified from frozen-thawed ovarian tissue may be a solution. This isolation procedure is routinely performed in our laboratory with healthy ovarian tissue, but it is not known if during isolated follicle pick-up, some malignant cells are taken that can contaminate the follicle suspension. By addressing this question, we aim to improve the technique to obtain completely disease-free follicles. Ten human ovarian cortical biopsies were enzymatically dissociated. A leukemic cell line was marked with a fluorescent cell tracer and added to the resulting ovarian cell suspension. The number of cells added was calculated based on vascular volume density of ovarian cortex. Retrieved follicles were divided into 2 groups based on pick-up technique used: (i) usual method; (ii) usual method plus washing follicles 3 times. The follicle suspension was investigated for the presence of leukemic cells using fluorescence microscopy. In the usual pick-up group, 221 leukemic cells were detected among 544 retrieved follicles, which was statistically different from the improved pick-up group, where no leukemic cells were detected among 707 retrieved follicles. In conclusion, this study proves that our usual follicle pick-up technique is not safe for leukemia patients. The improved technique is, however, highly effective at purging leukemic cells picked up along with follicles.