Assessment of reflectance confocal microscopy for non-invasive selection of optimal ovarian cortex fragments for autotransplantation.


ABSTRACT
RESEARCH QUESTION: Can reflectance confocal microscopy (RCM) be used to determine follicle density in human ovarian cortex fragments that are intended for fertility restoration? DESIGN: RCM was used on living cortex tissue fragments derived from five bovine ovaries and 13 human ovaries. All tissue fragments were cryopreserved and thawed before RCM analysis. Follicle numbers and distribution were determined by RCM and histology. Before and after RCM, general tissue viability and follicle integrity were assessed by a glucose uptake assay and neutral red staining, respectively. RESULTS: RCM can detect all stages of follicle development in living ovarian tissue to a maximum depth of 250 µm. In bovine tissue, all follicles were located within this 0-250 µm range. In human ovarian tissue, follicles were also present below the 250 µm RCM threshold, implying that only a percentage of the total number of follicles could be detected with RCM. The percentage of follicles detected by RCM appeared to be age dependent. The RCM procedure did not affect the glucose uptake by the tissue, whereas neutral red staining indicated a high level of follicle survival. CONCLUSION: In this proof of concept study, we have shown that RCM is a promising technique to determine the density of follicles ex vivo in living human ovarian cortex fragments, apparently without compromising the vitality of the tissue. Safety studies and further optimization of the RCM technique with a focus on increasing the penetration depth are required before clinical use of RCM.

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