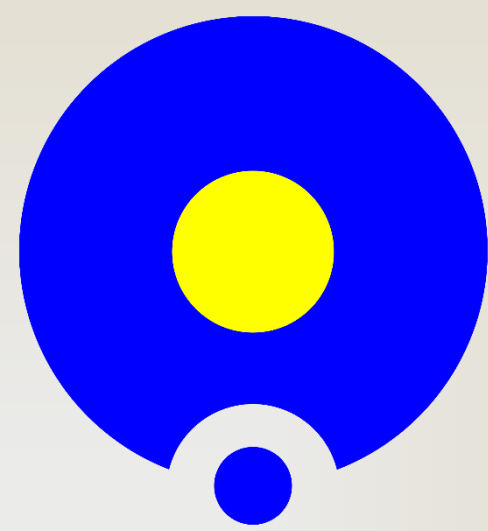


Optical coherence microscopy as a label-free tool for imaging live oocytes and embryos

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Introduction

Confocal laser microscopy and other fluorescence imaging methods applied in biology allow for detailed structural and dynamic studies of a single cell, but require fluorescent markers to visualize cellular architecture and may cause short- and long-term photo-damage. Bright field microscopy, although non-destructive, does not provide detailed structural information. **Optical coherence microscopy (OCM)** is a promising alternative, as it does not require sample pre-processing or labelling and is capable of providing 3D images of intracellular structures.

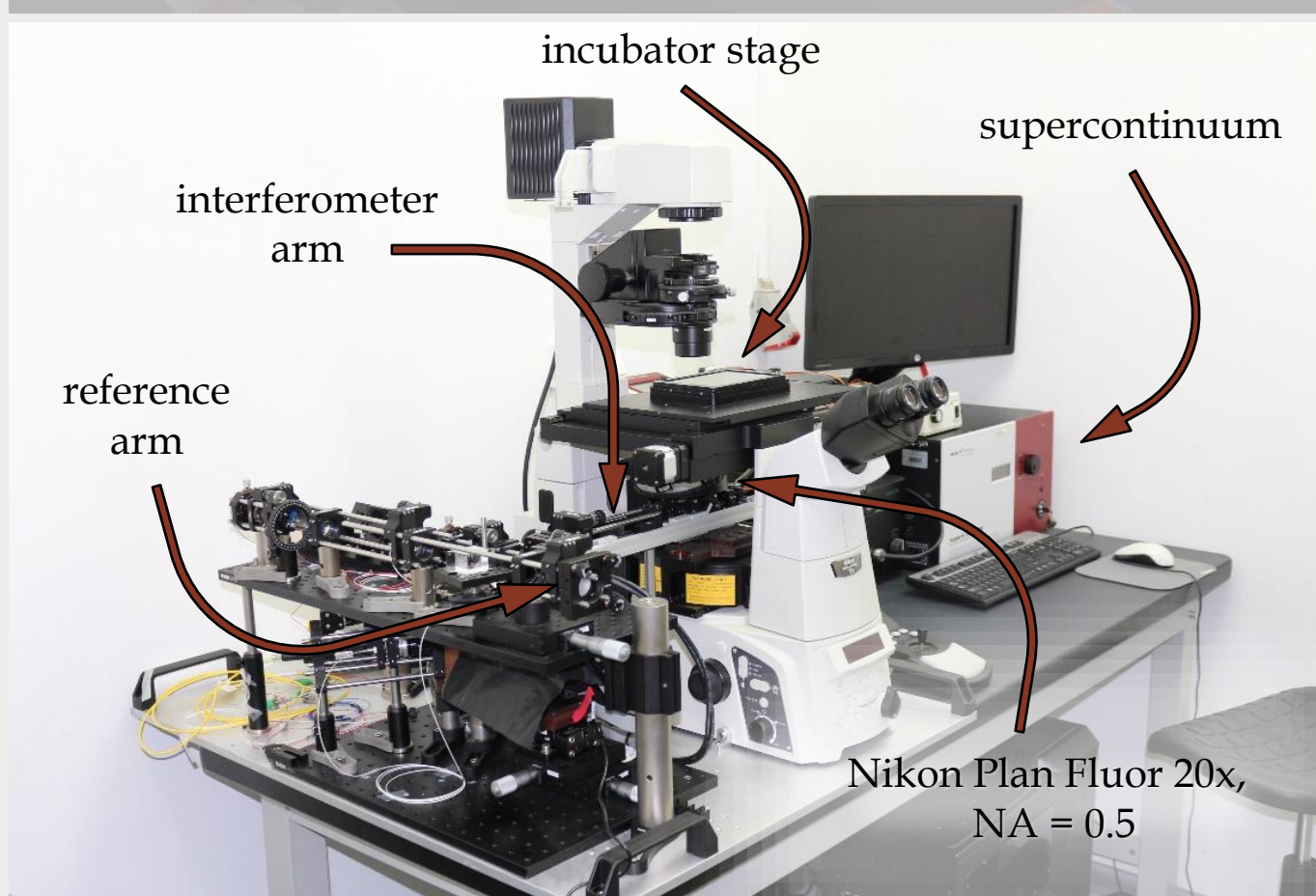
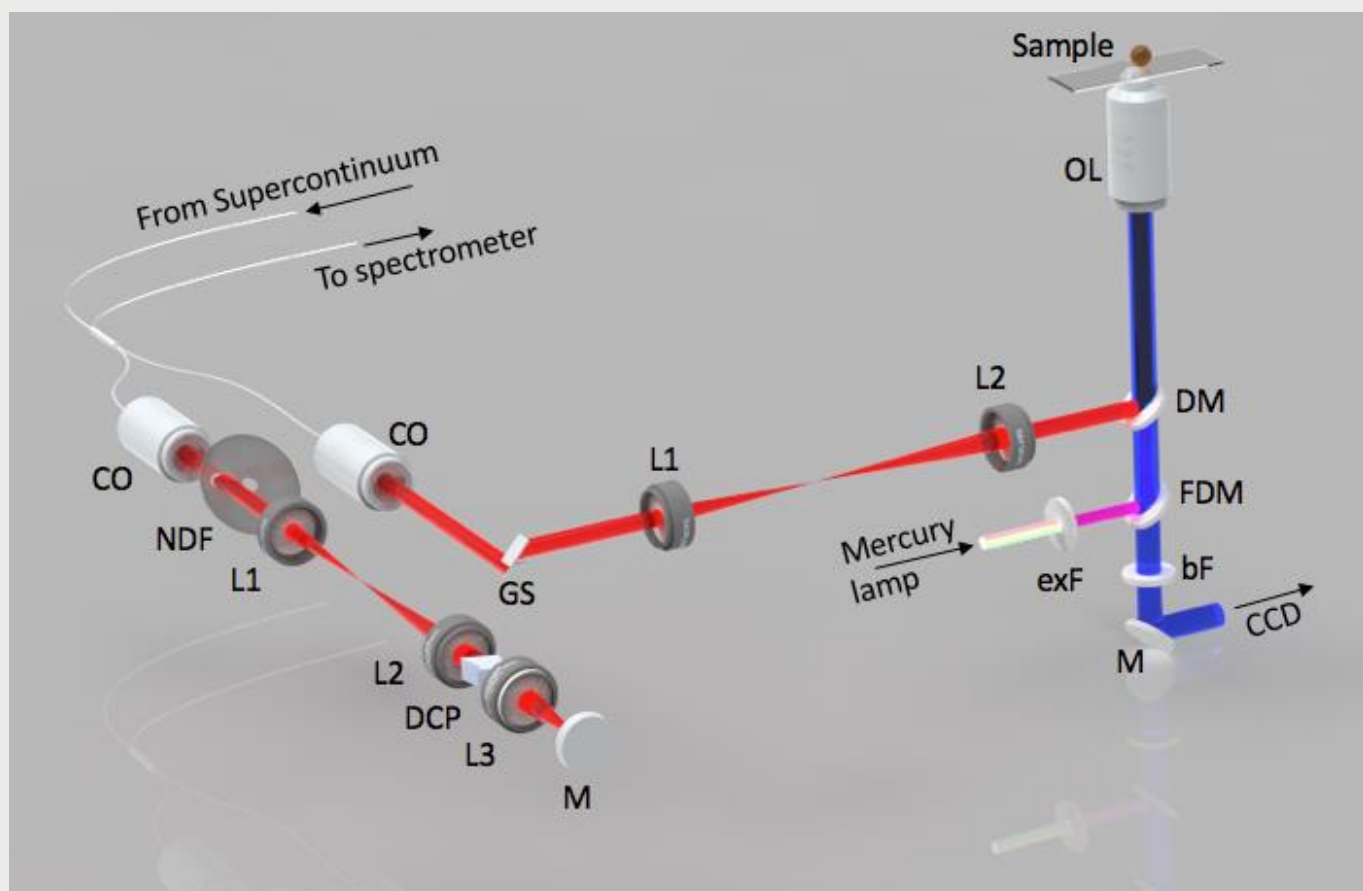
Here we show that images obtained by OCM provide information useful for oocyte and embryo quality assessment. We applied OCM to image chromatin arrangement in prophase I (GV) oocytes, allowing to distinguish transcriptionally active (NSN) and inactive (SN) oocytes. We have also shown that OCM is capable of visualizing the nuclei in compact morulas, allowing for cell count. These parameters correlate with the oocytes' and embryos' quality. The OCM scanning protocols were verified to be safe for oocytes and preimplantation embryos. Our results suggest that OCM may be a valuable addition to the imaging toolkit used in assisted reproduction procedures.

Objectives

- investigating whether OCM imaging affects oocyte maturation and embryo development;
- examining the relationship between the analyzed morphological parameters and the oocytes' and embryos' developmental potential in order to compile a predictive algorithm.

Methods

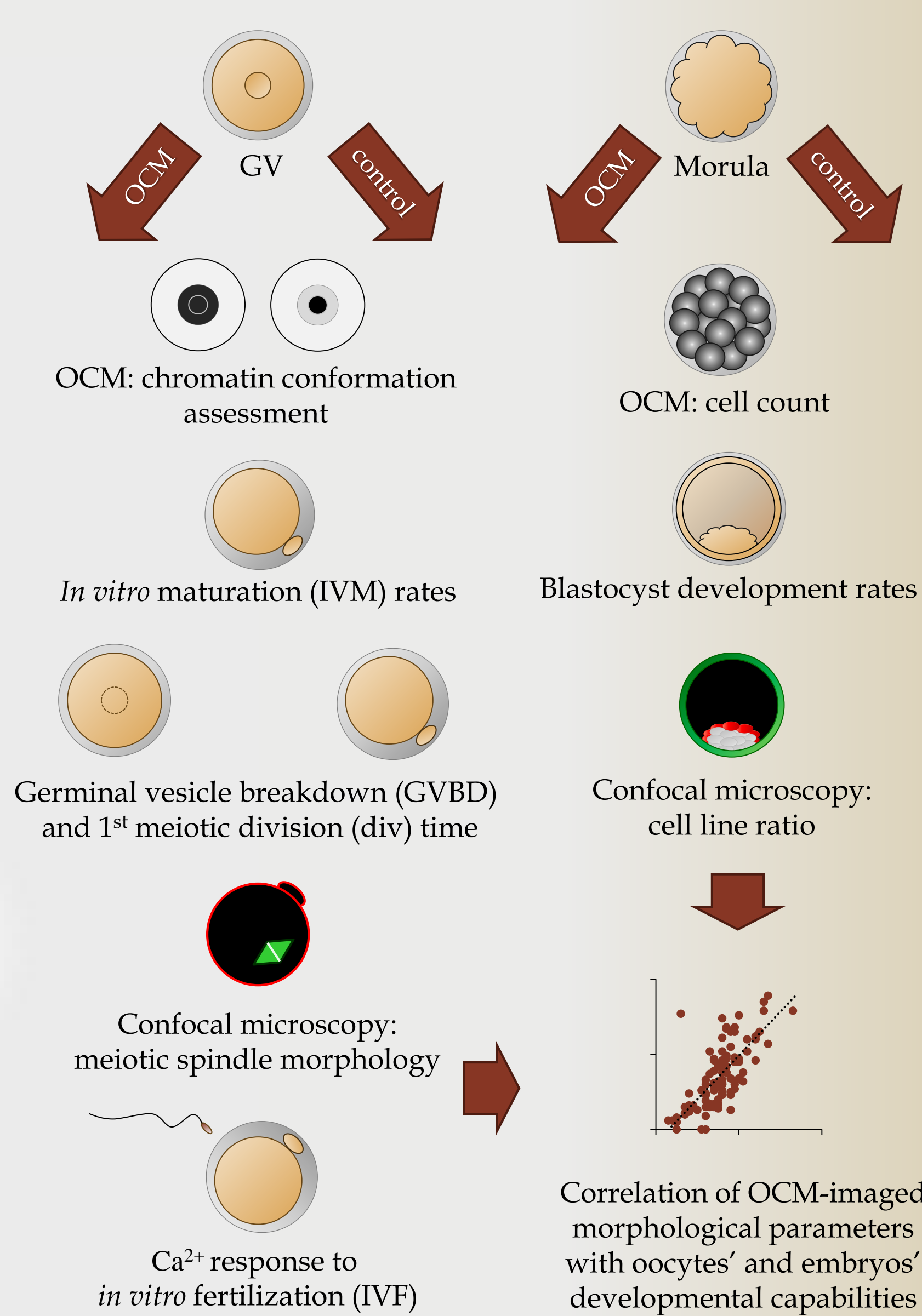
Schematic of the OCM imaging system



Experimental design

Oocytes and embryos scanned with OCM were cultured in order to compare their development with a non-scanned control group and to correlate the observed morphological parameters (chromatin configuration in case of GV oocytes and the nuclei number in case of morulas) with their developmental capabilities.

Experimental design outline



Results

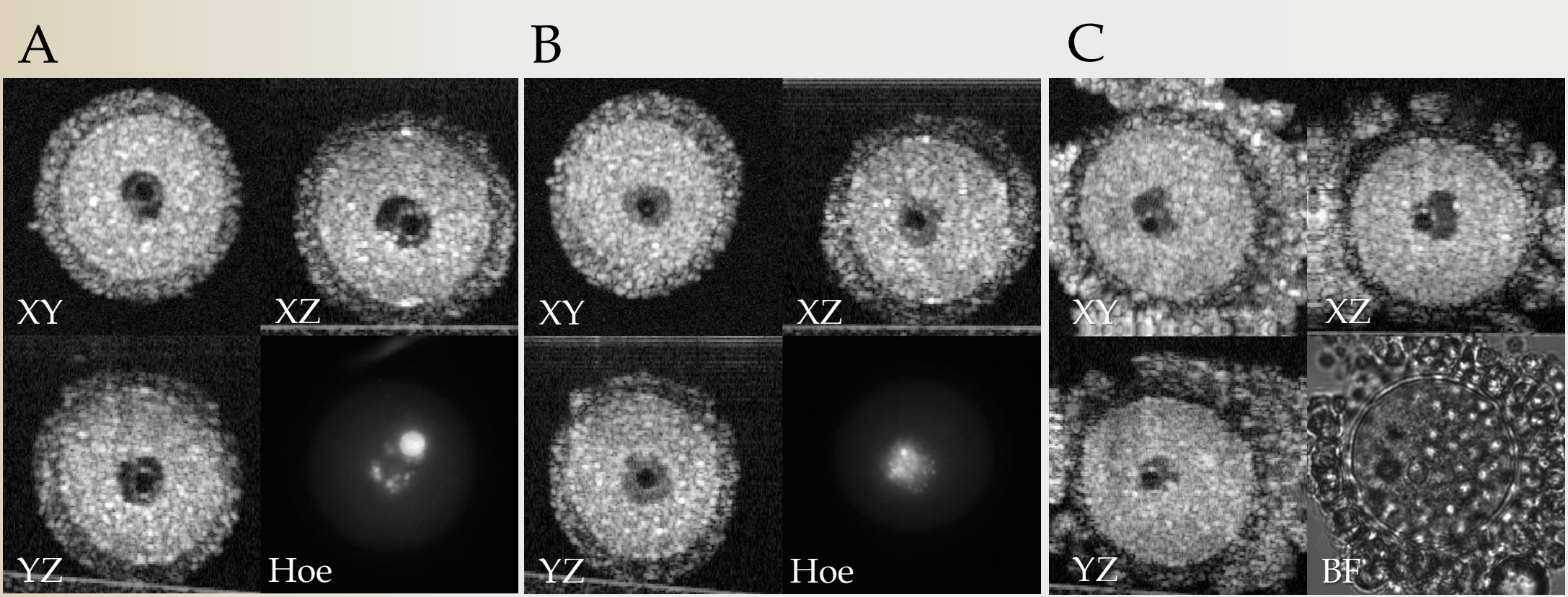


Fig. 1. OCM allows for chromatin conformation assessment in prophase I mouse oocytes. (A) SN and (B) NSN oocytes with 88% confidence vs. Hoechst 33342 staining ($r=0.92$, $n=24$ pairs); (C) Chromatin (NSN) conformation is visible in oocytes surrounded by granulosa cells.

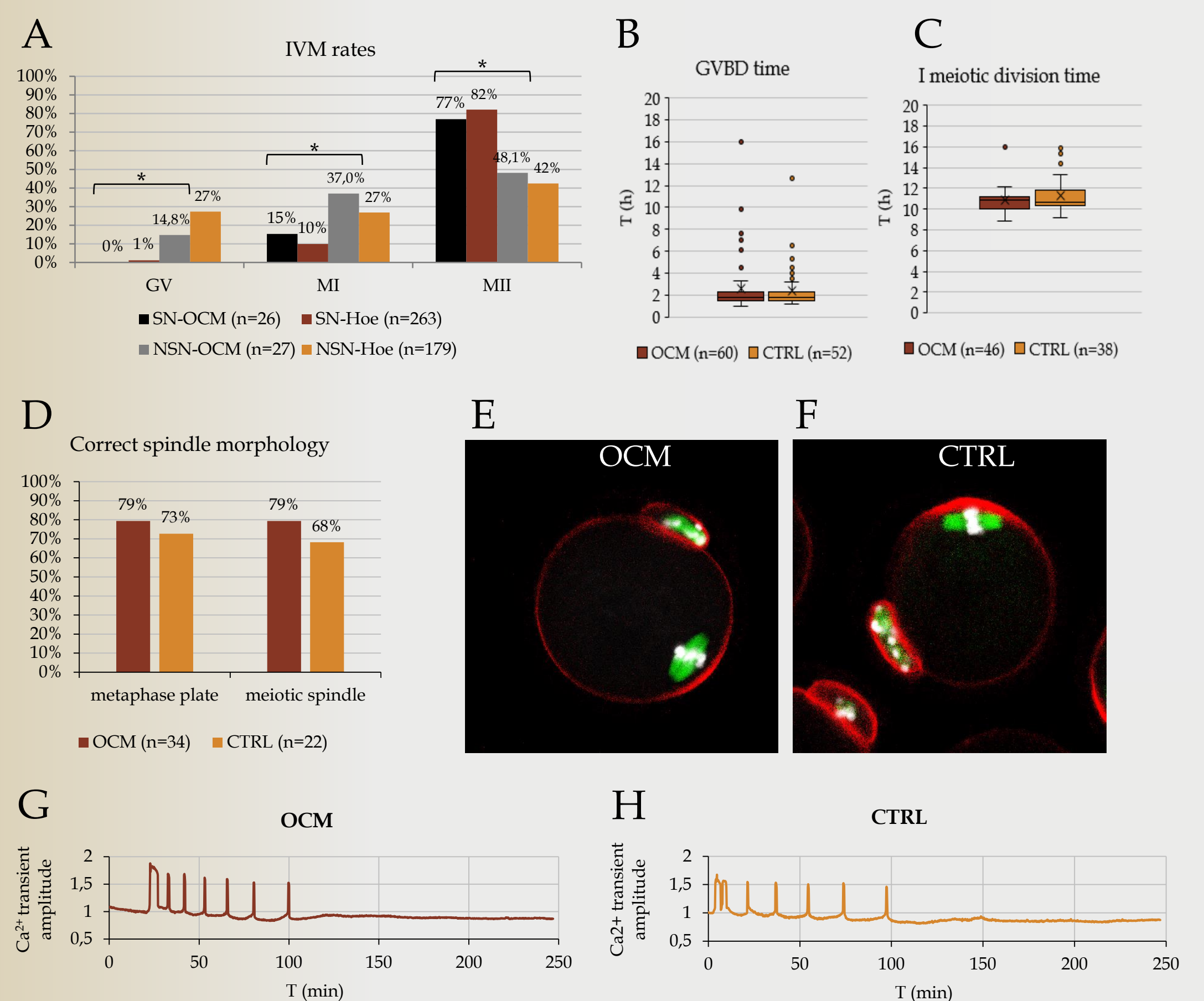


Fig. 2. OCM scanning does not affect GV's competence and allows for assessing their quality. (A) Oocytes marked as SN with OCM exhibit higher competence to complete meiosis compared to NSN ($p<0.05$) – similarly to the control (Hoechst-stained) group ($p>0.05$). (B-C) GVBD and 1st meiotic division time for OCM-scanned and control (non-scanned) oocytes are similar ($p>0.05$). (D) In vitro matured eggs display similar rates of correct spindle morphology in (E) OCM-scanned and (F) control groups ($p>0.05$). (G-H) Representative $[Ca^{2+}]_i$ oscillations at fertilization patterns for MII oocytes derived from OCM-scanned ($n=21$) and control ($n=24$) GVs. The readings are normal ($p>0.05$).

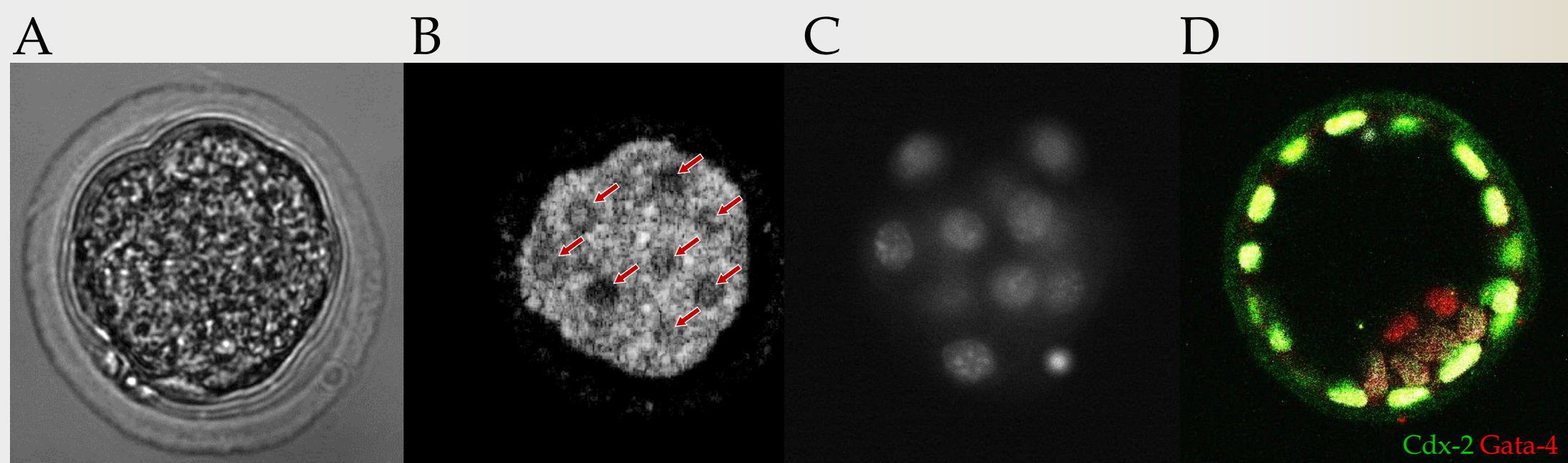


Fig. 3. OCM-rendered nuclei count is a useful tool for assessing embryos' developmental potential. (A) Bright field image of a morula-stage embryo; (B) OCM scan of the same E3.0 embryo with visible nuclei (arrows); (C) Fluorescence microscopy image of E3.0 embryo with Hoechst-stained nuclei to confirm the cell count ($r=0.97$, $n=30$ pairs) (D) confocal scan of E4.0 blastocyst derived from OCM-scanned E3.0 embryo. (E) The number of cells observed with OCM at E3.0 correlates with the total number of cells registered with confocal microscopy in E4.0 (Spearman's $\rho = 0.75$, $n=103$ pairs).

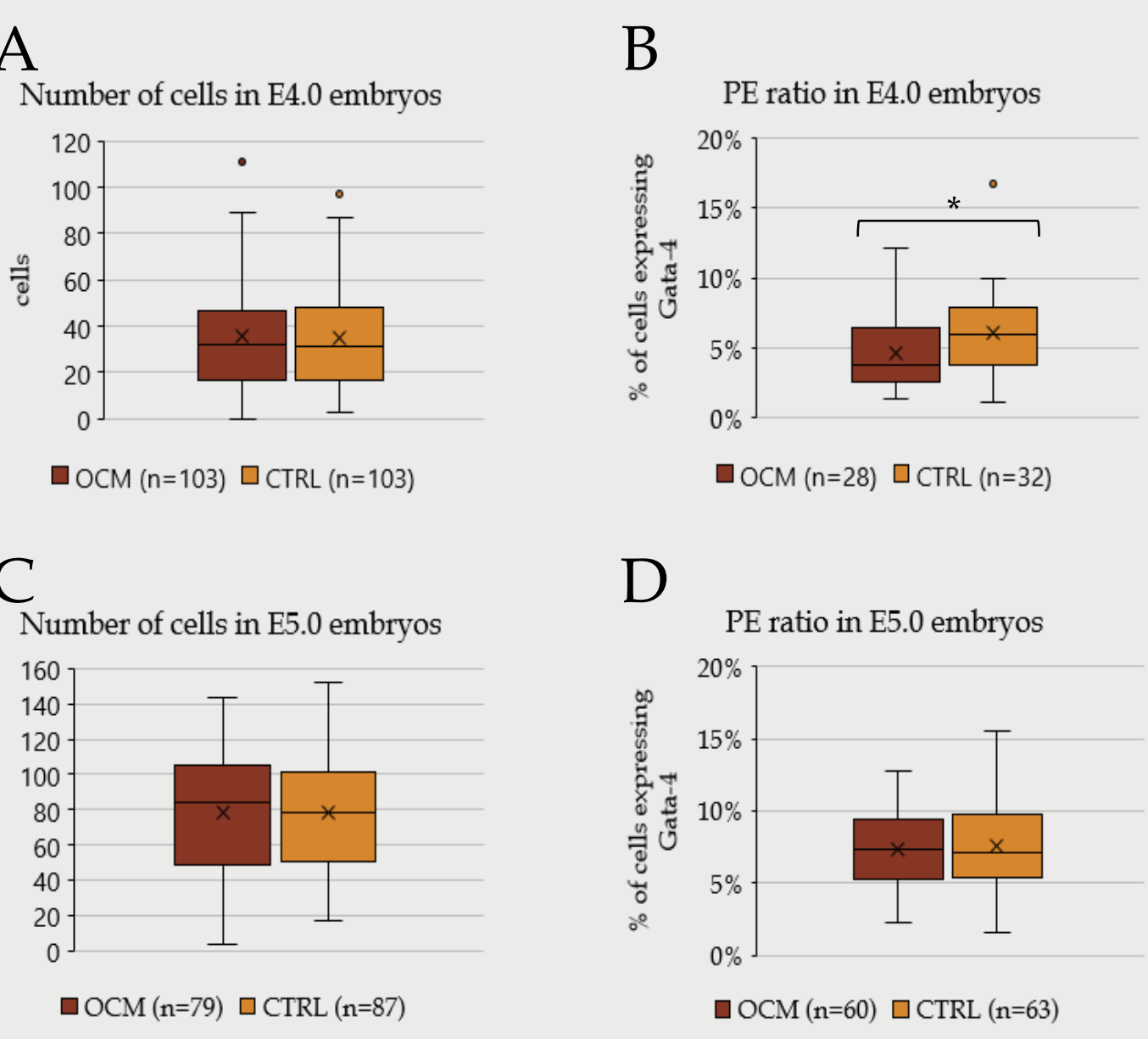


Fig. 4. OCM scanning is safe for mouse embryos. Total cell count in (A) E4.0 and (D) E5.0 OCM-scanned and control embryos; (B) OCM-scanned embryos display a slightly lower ratio of PE at E4.0 compared to control embryos ($p<0.05$) (E) but the differences disperse at E5.0 stage ($p>0.05$), suggesting a minor delay in PE differentiation that is rectified by the implantation stage.

Conclusions

- OCM allows for 3D **non-invasive** imaging of mammalian oocytes and embryos, allowing for **quality assessment**, and therefore can be potentially useful for clinical applications, e.g. oocyte/embryo selection in IVF clinics.

Bibliography

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