

## **EGG MATURITY ASSESSMENT USING POLARIZED LIGHT MICROSCOPY HELPS TO PREVENT PREMATURE FERTILIZATION OF LATE MATURING OOCYTES.**

Holubcová, Zuzana<sup>1,2</sup>; Kyjovska, Drahomíra<sup>2</sup>; Martonova, Martina<sup>2</sup>; Paralova, Darja<sup>2</sup>; Klenkova, Tereza<sup>2</sup>; Otevrel, Pavel<sup>2</sup>; Hampl, Ales<sup>1</sup>

<sup>1</sup>Masaryk University, Faculty of Medicine, Department of Histology and Embryology, <sup>2</sup>Reprofit International

Presence of polar body (PB) together with metaphase II (MII) spindle indicates completion of oocyte maturation and its readiness for fertilization. However, in human oocytes, PB emission considerably precedes bipolar spindle formation. Nevertheless, in clinical practice, all PB-displaying oocytes are assumed as mature and subjected to the intracytoplasmic sperm injection (ICSI). This puts oocytes completing development in vitro at the risk of untimely sperm entry. We have taken advantage of the polarized light microscopy (PLM) and birefringence properties of the meiotic spindle to assess egg maturity and optimize the timing of ICSI with respect to the development stage of the oocyte. Total of 918 eggs from 231 patients with suboptimal response to hormonal stimulation (<6 MII oocytes retrieved) was subjected to non-invasive spindle imaging prior to ICSI. PLM examination revealed that the spindle signal was absent in 32.57 % of PB-displaying oocytes. This was not unexpected given that 55.99 % of analyzed oocytes were immature at the time of retrieval but extruded PB before scheduled ICSI. In cases with a majority of spindle-negative oocytes, ICSI was deliberately postponed and performed immediately after second PLM examination ~2 hours later. Here, 59.39 % of initially spindle-negative oocytes displayed spindle birefringence during second PLM assessment. Our data confirmed higher fertilization and developmental potential of spindle-positive eggs reported previously (utilization 44.97 % vs. 18.81 %). All 33 live births reported in this study resulted from embryo transfers involving spindle-positive oocytes. Noteworthy, 7 babies were conceived by fertilization of in vitro matured oocytes which are typically discarded. In conclusion, we have demonstrated that postponing ICSI of late maturing oocytes allows for bipolar spindle appearance associated with better clinical outcomes. Avoiding premature fertilization is particularly important in cycles with a low number of MII oocytes available for ICSI and could make a difference for poor prognosis IVF patients.