

NON-INVASIVE ASSESSMENT OF OOCYTES AND EMBRYOS: IMAGING

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Light microscopic imaging has been used and still is in use to investigate morphology and structure of human oocytes and embryos. Gross morphological deficiencies do correlate with success in in-vitro fertilization (IVF) programme, however, normal light microscopy does allow a conclusion on the implantation potential if all oocytes / embryos do look alike.

Recently, a new type of polarization microscope system was developed and used in the field of ART for imaging of birefringent structures present in the oocyte, namely the spindle and the zona pellucida.

Spindle imaging: Only an optimal set-up of the incubators, microscopic systems and handling steps will allow controlling essential factors like temperature and pH which do influence the visibility of the spindle. Therefore the temperature and pH sensitivity of spindle imaging seems to be a good tool for quality control. In laboratories with reduced visualization of spindles in living human oocytes, the laboratory conditions should be carefully monitored.

The presence of the spindle in a mature oocyte can predict fertilization rates, cleavage rates and embryo quality, whereas the outcome in terms of pregnancy and implantation rates is not yet proven. A major aspect of spindle imaging is the dynamics of the spindle in the meiotic transition from the germinal vesicle stage to the metaphase-II-oocyte. In this sense, timing of spindle imaging is of utmost clinical importance. Oocytes classified as metaphase II based on the presence of a first polar body by conventional light microscopy, maybe in early telophase I with spindle remnants linking the polar body and the ooplasm as seen by polarization microscopy. If such an oocyte is injected with a spermatozoon, it will be activated by the sperm but at a non-physiological time point and show three pronuclei on the next day. Therefore, a single observation of the spindle appears to be inadequate to identify if an oocyte without a visible spindle is abnormal (absence of spindle) or has just entered late telophase I. Besides the location of the spindle one can also investigate spindle retardance, which is directly proportional to the density of the microtubules. However, spindles are dynamic structures and spindle retardance measurements are orientation dependent and an increase in spindle retardance can be observed during certain physiological events like oocyte activation. Therefore, the use of spindle retardance measurements as a predictive tool is still under debate.

Zona imaging: Besides the visualization of the spindle, zona imaging was proposed as another valuable predictive marker of oocyte / embryo quality. Assessment of the zona pellucida by conventional microscopy cannot be used as a predictive factor for the success of ICSI. However, polarisation microscopy allows the distinction of three layers within the zona pellucida of human oocytes. The inner zona layer exhibits the highest amount of birefringence. In contrast to spindle imaging, zona imaging is more suitable for an automatic sampling of measurement values allowing an objective and user-independent scoring of the corresponding oocyte. Two devices are at present available which allow for automatic zona imaging. One such measuring device is based on the automatic detection of the birefringence of the inner zona layer. Once detected, a software module automatically starts to calculate and display in real-time a zona-score based on the intensity and distribution of the birefringence at 180 measuring points. Several studies have shown that zona birefringence is related to the potential of an embryo to develop to the blastocyst stage and hence to success in ART. Even zona imaging based selection of oocytes for further culture and transfer does lead to better implantation and pregnancy rates.

The ratio for zona imaging is the paracrystalline network structure of the zona pellucida which is build up by the oocyte during the maturation process. A high and uniform birefringence of the inner zona layer is indicates a regular structural integrity of the zona pellucida and may reflect an optimal cytoplasmic potential of an oocyte resulting in better developmental competence for embryonic growth and implantation. Therefore, zona imaging is a good prognostic factor of oocyte and embryo viability, provided that the evaluation is performed at the oocyte stage because zona birefringence increases with prolonged culture This complex deserves further attention and research.

Conclusions: Birefringence imaging of the spindle and of the inner layer of the zona pellucida is a new tool in assisted reproduction techniques. Data of numerous studies indicate that birefringence imaging can improve the selection of oocytes which can develop into embryos with high viability. The technique is non-invasive and can easily be applied in an IVF programme. For certain applications, like zona imaging, a single observation can significantly improve oocyte selection and consecutive implantation and pregnancy rates. Birefringence imaging may proof to be a valuable add-on technique for sampling as much information as possible to identify the oocyte which has the highest chance to develop into an implantation competent embryo. It is another step towards achieving a pregnancy following single embryo transfer. The spread of this technique will soon result in new applications and new insights in basic research as well as in clinical assisted reproduction.