aneuploid. In the OAT group, an average of ~200 sperm nuclei per patient were analysed. In this group, the total frequency of aneuploidy for chromosomes 18, X and Y was 7.4% (range 2.6–11%). Sex chromosome abnormalities were observed in 4.9% of the sperm nuclei. No relationship was observed with respect to the total number of vital sperm cells (range 0.3–4.6 × 10^6) or with respect to the sperm morphology (range 0–15% normal sperm cells, according to World Health Organization criteria) in the ejaculates used.

**Conclusion:** The incidence of aneuploidy is increased in prepared semen samples of infertile men with very poor semen parameters and is mainly due to the increase in sex chromosome abnormalities. However, larger numbers of cells need to be analysed to confirm these data. It is interesting to speculate that the increased incidence of sex chromosome abnormalities in ICSI offspring is due to the increased sex chromosome aneuploidy in the sperm nuclei of men with OAT.

---

**Ultrastructure of human fertilization after intracytoplasmic sperm injection**

Stefania A. Nottola¹, Sayoko Makabe², Guido Macchiarelli¹, Giuseppe Familiari¹, Cristina Verlengia¹, Silvia Correr¹ and Pietro M. Motta¹

¹Department of Anatomy, University La Sapienza, Rome, Italy and ²Department of Obstetrics and Gynaecology, Toho University, Tokyo, Japan

**Introduction:** The objective of this study was to evaluate a series of ultrastructural parameters in pronuclear and early cleaving (2- to 4-cell stage) human eggs fertilized after ICSI. These data were compared further with previous information on eggs deriving from conventional IVF or sampled from the oviduct.

**Materials and methods:** Surplus samples (from syngamy to 4-cell stage) were obtained after patients (n = 11) had given informed consent, and these eggs were subjected to an ICSI protocol. A GnRHa–HMG–HCG protocol was used to induce superovulation in these patients. Observations were performed by scanning and transmission electron microscopy (SEM and TEM).

**Results:** By SEM the zona pellucida (ZP) investing pronuclear as well as cleaving fertilized eggs showed a spongy texture, with large or smaller meshes. This three-dimensional architecture of the ZP is similar to that observed in healthy, mature oocytes and IVF eggs after fertilization. A small hole (1–1.5 μm) with a regular, sharp border was found on the ZP surface of a 4-cell egg. The area around the hole appeared intact and regularly meshed. This hole may represent the surface marker of the pre-existing larger site of sperm injection. The detection of such a hole should be considered an exceptional finding, particularly at the 4-cell stage. In fact, as recently reported, the ZP, thanks to its elastic properties, seems usually to undergo prompt and complete repair within 15 min after microinjection. By TEM, pronuclear eggs appeared as large, rounded cells, with regular pronuclei containing dense nucleoli, and with abundant mitochondria–vesicles complexes scattered in the cytoplasm. The mitochondria
were rounded and had arched cristae. Numerous microvilli projected into a narrow perivitelline space. Two- to 4-cell eggs showed similarly sized nucleated blastomeres, often associated with small cell fragments. One to six nucleolar precursors and fragments of annulate lamellae were seen in the nucleoplasm. Mitochondria–vesicles complexes, still abundant in 2-cell eggs, became smaller and less numerous in 4-cell eggs. A large smooth endoplasmic reticulum aggregate, probably a remnant of the ‘microinjected sperm-associated tubular smooth endoplasmic reticulum’ recently described in human oocytes unfertilized after ICSI, was found near the nucleus of a 4-cell egg blastomere. Membranes and vesicles belonging to Golgi complex were also seen. Neither communicative nor adhesive junctions were found between blastomeres.

Conclusions: The structures we have evaluated so far have generally appeared well preserved. Further, the data hereby reported (including the presence of fragments associated with normal blastomeres) are similar to those previously obtained with IVF material, even presenting the same peculiarities that IVF samples have shown, compared with in-vivo fertilized eggs. Therefore, we conclude that ICSI does not significantly affect egg ultrastructure.