

ELECTRON MICROSCOPY FOR DIAGNOSIS OF MALE INFERTILITY

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Intracytoplasmic sperm injection (ICSI) is considered the most effective existing treatment for various kinds of male factor infertility [1]. Many authors agree that an improvement in clinical management can be achieved by expansion of semen quality analysis. Sperm morphology assessment according to strict criteria is widely accepted to be a valuable and stable test for predicting the “*in vivo*” [2] and “*in vitro*” [3] sperm fertilizing capacity. Light microscopy (LM) cannot recognize the entire variety of morphological defects. In contrast, transmission electron microscopy (TEM) allows identifying the ultramorphological condition of the sperm cell organelles and thus determining a more specific sperm quality [4]. Recent evidence has indicated that in many of infertile patients a genetic component is present and that depending on the nature of sperm pathologies, the outcome of “*in vitro*” fertilization (IVF) versus ICSI could change considerably [5].

The aim of this study was to correlate the information obtained from LM with that using TEM from patients suffering severely alterations of semen parameters. By this method we are trying to find systematic alterations that tend to show family clustering and have probably genetic origin. [5].

Men between 29 and 38 years old complaining of primary infertility were included. Three fertile men were included as controls. Past history and complete andrological examination provide no information on clinical problems. The semen was obtained by masturbation and studied under phase contrast microscopy. Motility and viability were investigated according to standard methods [6]. LM morphology was evaluated according to Kruger’s criteria [4]. We have then chosen those samples that LM revealed severe alterations in sperm motility and morphology. When liquefaction was complete, the samples were diluted (1:3) with phosphate buffer and spermatozoa were separated by centrifugation. The pellets were fixed with 4% paraformaldehyde with 2,5 % glutaraldehyde in 0.1M phosphate buffer (pH 7.4) at 4°C over night, and post fixed in 1 % osmium tetroxide in the same buffer for 30-60 minutes at room temperature. The samples were dehydrated in a graded ethyl alcohol series, followed by three changes of anhydro acetone, and embedded in a hard viscosity Araldite resin. The blocks were cut in a RMC MT-X ultramicrotome with a DIATOME diamond knife, and the ultrathin sections were double stained with uranyl acetate and lead citrate and examined in a JEOL JEM-1010 TEM at 80-kV accelerating voltage. The evaluation of the samples was performed on 100 randomly selected sperm cells and each specific ultramorphological characteristic was documented and quantified.

The sperm cells analyzed showed severely damaged nucleus where the compact arrangement of the chromatin is replaced by empty areas that occupy high percentage of the nucleus. The characteristic 9+2 microtubular structure of the flagellum presented high degree of disorganization, absence of the two central microtubules and transposed of the external microtubules. Distortion of the fibrous sheath was also observed. On the other hand the connecting piece including the centriolo looked normal. Some of the ultrastructural characteristics found are shown in the figure.

We concluded that TEM could be an excellent tool to classified semen samples in order to better identify sperm pathologies. This technique could lead the following studies and help to decide if immunohistochemistry or genetics analysis should be performed. In this study the samples analyzed by TEM suggested both chromatin and axonema defects. Efforts should be focused on detecting if there are specific genes that can explain those phenotypes.

References

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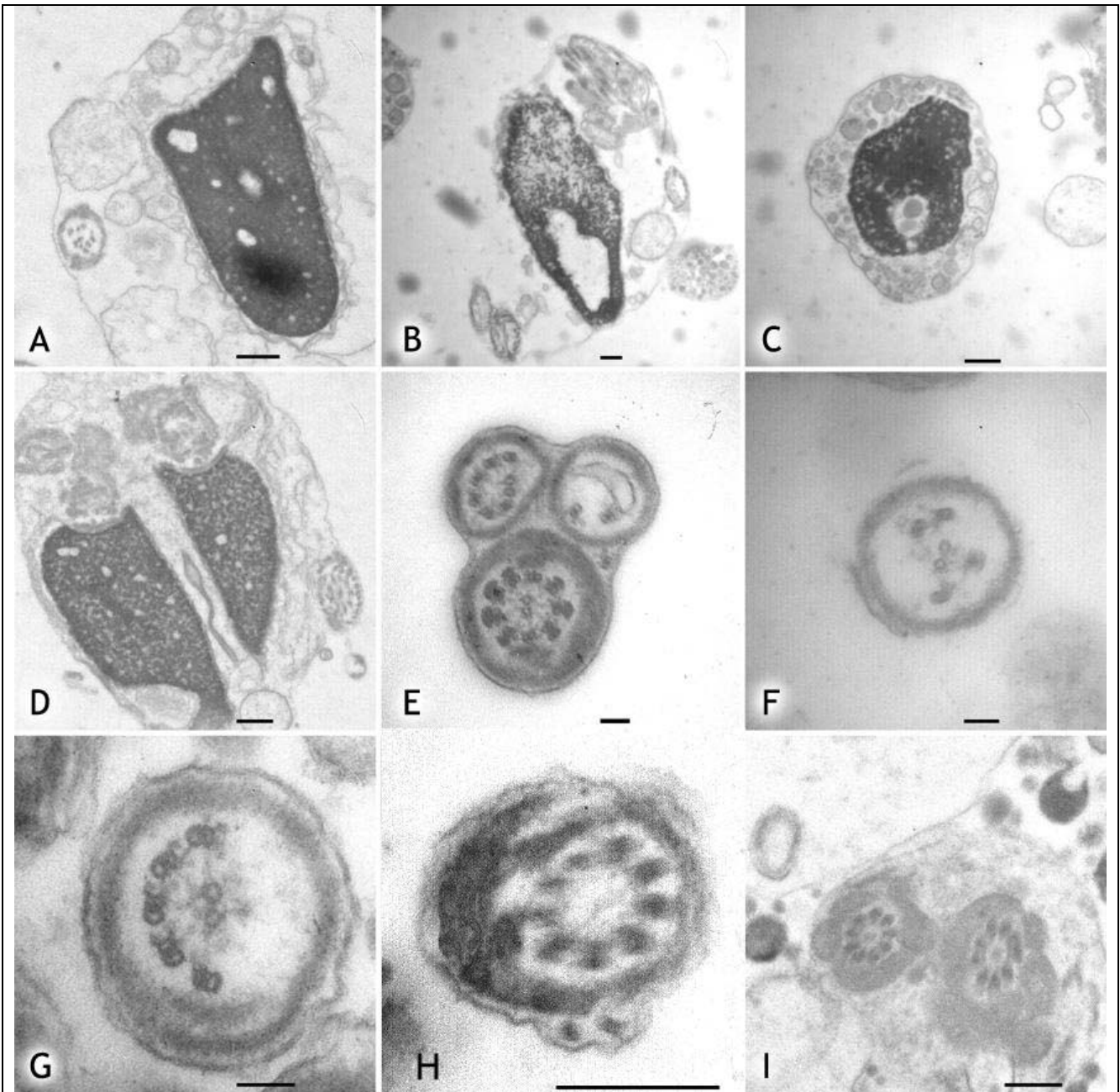


Figure. Sperm ultrastructural characteristics: (A-D) altered nuclei with uncondensed chromatin, (E-F) transposed or absence of the external microtubules, (H) absence of the central pair, (I) section of two normal sperm. Scale bars represent (A-D) 0.5 μ m, (E-G) 0.1 μ m, (H-I) 0.5 μ m.