

All-round approach in diagnosis

Ashok Agarwal¹, Chak-Lam Cho², Sandro C. Esteves³, Ahmad Majzoub⁴

¹American Center for Reproductive Medicine, Cleveland Clinic, Cleveland, OH, USA; ²Division of Urology, Department of Surgery, Kwong Wah Hospital, Hong Kong, China; ³ANDROFERT, Andrology and Human Reproduction Clinic, Referral Center for Male Re-production, Campinas, SP, Brazil; ⁴Department of Urology, Hamad Medical Corporation, Doha, Qatar

Correspondence to: Ashok Agarwal. Professor and Director, American Center for Reproductive Medicine, Cleveland Clinic, Mail Code X-11, 10681 Carnegie Avenue, Cleveland, OH 44195, USA. Email: AGARWAA@ccf.org.

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Dr. Veeramachaneni (1) in response to the practice recommendations by Agarwal *et al.* (2) reminded the importance of interacting multiple factors on reproductive system. Indeed, it is of paramount importance to rule out reversible factors (such as genital tract infection and exposure to environmental toxicants) during the assessment and management of infertile patients. In contrary to the notion of one-man one-disease in traditional teaching, multiple intercalating factors are often present in a couple investigated for infertility (3). This is one of the reasons for the standardization of semen sample collection before any test in order to minimize the impact of confounding factors (4). These include possible exposure to heat stress, drugs and environmental toxins which should be ruled out in the history taking before clinical tests. Symptoms or microscopic features (e.g., leukocytospermia) suggestive of genitourinary tract infection warrants further investigation and correction (5).

Dr. Veeramachaneni enlightened the reader on his extensive experience in utilizing light and transmission electron microscopic evaluation on semen samples (1). We wish to discuss and comment on this important technique. The principle of transmission electron microscopy (TEM) addresses the deficiency of routine light microscopy in characterization of subtle lesions in spermatozoal organelles and cellular debris in the semen. Indeed, TEM has been used extensively in studies of immotile human spermatozoa (6). TEM is potentially useful for investigation of severely asthenozoospermic patients. The stratification

of patients by different TEM features, i.e., dysplasia of the fibrous sheath or non-specific flagellar anomaly, points to different etiologies and prognosis in pregnancy outcome (7). It is, therefore, postulated that this approach allows assessment of seminal components and characterization of pathologic conditions in the genital tract (8). Although structure and function may correlate to a certain extent, not all structurally normal spermatozoa have normal function and vice versa. Experience with sperm DNA fragmentation (SDF) testing has shown that sperm with high DNA fragmentation can have normal motility and morphology (9). In addition, intra-individual variations is a major concern during assessment of any sort of semen characteristics/parameters. Similarly, the inter-observer variations in the results of TEM may be significant since the technique requires high level of expertise in cytological assessment. The method of TEM may have the same drawback as semen analysis in variations between laboratories and in an individual over the course of time (10). On the other hand, the validity of a single Sperm Chromatin Structure Assay (SCSA) analysis has been shown to have a high predictive value for assessment of fertility *in vivo* (11). As suggested by Dr. Veeramachaneni, cytological technique may possibly characterize the pattern of SDF which may point to different etiologies (1). However, a particular pattern of ultrastructural changes in semen may more likely point to a common mechanism rather than revealing pathognomonic features specifically suggestive of a diagnosis. Further studies are required to correlate

cytological features under TEM and underlying etiology. The presence of SDF as suggested by ultrastructural change may not imply the presence of pathological conditions since a certain level of SDF is present in normal fertile individuals (12). Although visualization of pattern of SDF was suggested by the author as a potential diagnostic tool, more data is needed in determining its exact role.

The technique of TEM has been studied mainly in animal models (13). However, its utility in clinical practice must be supported by human data. Many of the studies were conducted with a relatively short duration of single-agent exposure to toxin (8). The result may not be applicable to clinical practice in human when chronic low-dose exposure to multiple environmental toxins is commonplace. The degree of implication of ultrastructural alterations in sperm and semen is unknown. Further studies in correlating TEM findings with reproductive outcomes, e.g., fertilization rate and embryo quality, will be useful. Nonetheless, TEM study on semen possibly provides a non-invasive alternative to assessment of infertile male. The ability to assess desquamated germ cells, denuded Sertoli cells and epithelial fragments from excurrent ducts and accessory glands is unique among currently available technologies. Preliminary data demonstrating the ability to diagnose intratubular germ cell neoplasia in a non-invasive manner is attractive since there is a relationship between infertility and testicular malignancy (14).

We realize that every clinical test has its strengths and weaknesses and there is room for refinement of both SDF testing and TEM study alike. The encouraging preliminary findings of laboratory tests in the bench require verification in human studies to elucidate their role in clinics. In the context of complex reproductive system, a single test with clear cut-off values is probably not available (15). We trust that a comprehensive panel of analyses, including both structural and functional assays, are needed for the full assessment of patients.

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Footnote

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