Practice Recommendations of Sperm DNA Fragmentation Testing: Expert Commentaries by Invited Authors and Replies by Guest Editors

Contributors from Oceania

Commentary

Assessing the male in fertility clinics—men undervalued, undermanaged and undertreated

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Having commenced a specialist career focused on resolving infertility for couples since 1976 (JLY), the following issues have firmed over the 40 years:

- Infertility arises as an issue for "couples", meaning the majority of cases have identifiable issues from both partners.
- Such couples should be managed together in a Fertility Clinic which embraces the full gamut of assisted reproductive technologies (ART) including intra-uterine insemination (IUI) and *in vitro* fertilisation (IVF) along with an integrated Andrology service.
- The interaction of the identifiable fertility factors is complex and not fully clarified despite the phenomenal advances in knowledge concerning reproduction over this period.
- There is an awakening that the factors impacting on the fertilization of gametes probably has an equivalent likelihood from the perspective of both the male (spermatozoa) as well as the female (oocytes), although the dynamic processes affecting spermatogenesis and oogenesis are very different.
- Despite this acknowledgement, the majority of Fertility Clinics worldwide remain focused on the female who always gets a thorough clinical evaluation as well as screening tests and the consideration of numerous treatment options; whereas similar focus for the male generally occurs only when a semen analysis is severely "abnormal".
- The evolution of IVF provided opportunity for pregnancies despite some degree of "male factor" infertility (1), but the process of intracytoplasmic sperm injection (ICSI) curtailed clinical research on the male aspects, almost completely.

Clinical Andrology—a current weakness in modern fertility management

In the pre-ICSI era, we searched for ideas to enhance spermatozoal function to increase the chance of fertilization and focused on tests such as acrosome reaction to ionophore challenge (ARIC) (2) which guided us, for example in the use of pentoxifylline (3) to enhance sperm motility, assist the acrosome reaction and counter reactive oxygen species

(ROS) (4). We even explored mechanical methods to assist "weak" sperm to get to the oocyte by techniques such as partial zona dissection (PZD) and subzonal injection (SUZI) (5). Some of us took expansive histories from the male partner, conducted clinical examinations and even performed ultrasound examinations as an investigative routine (6); hoping to become more enlightened about the underlying causes for a couple failing to conceive.

In the current ICSI era, many infertility clinics have reduced their vigilance over the male, although never reducing such over the female partner, mainly because she endures the procedures and carries the pregnancy. Female screening remains important to exclude non-fertility pathologies and medical disorders relevant for undertaking procedures and managing the ensuing pregnancies. Of course, female patients must be examined to check on uterine anatomy, determine the antral follicle count (AFC), and analyse hormones including the popular anti-mullerian hormone (AMH) level (7). Otherwise the evolution of assisted reproduction is increasingly focused on the cellular aspects and intracellular functions to improve embryo quality. From the male perspective, semen analysis has been a minimal contributor. Some suggest that the tests simply provide a yes or no answer about the presence of sperm in the ejaculate; or provide minimal contribution to qualitative considerations, only partially improved by more intensive evaluation such as the focus on strict morphological criteria (8). Such was probably best provided by the hemi-zona assay (HZA) (9) whereby sperm binding on the same zona pellucida was compared between proven control and patient sperm samples. The denuded hamster oocyte penetration test (HOPT) (10) was an attempt to rise above the deficiency of semen analysis and even provide a guide for which varicoceles might benefit from ligation. These labour-intensive tests disappeared once ICSI became widely established.

However, ICSI does not provide us with a clear understanding of the nature of infertility, other than recognizing that there are age-related issues affecting both partners along with probably hundreds of other variables. For example, world-wide our environment appears to becoming increasingly toxic with estrogenic compounds causing endocrine disruption (11). We struggle to understand the relevance of clinical findings such as endometriosis, fibroids and adenomyosis in the female and varicocele in the male or the several oddities noted on genitourinary ultrasound, including microlithiasis and cystic ectasia of the rete testis (6,12). Earlier medical texts indicated a reflex response to surgery was indicated for varicocele, but the evolution of ideas from Archie Cochrane and elevated standards of Evidence-Based Medicine (EBM) (13), has left us without a straight-forward therapeutic pathway. The story of varicocele is the best example of our current confusion with no less than three Cochrane reviews condemning surgery as useless, but the emergent data now condescending that there might be a benefit (13) and that conclusion being drawn without including the impressive results of microsurgical inguinal or sub-inguinal surgery reported by Surgical Andrologists from Cornell-Weill (14,15).

Re-integrating comprehensive male management

From this pre-amble, clearly the field seeks answers concerning the correct pathway. We suggest the following:

- Universally re-employ essential medical practice; including expansive historical information along with clinical evaluation, appropriate screening tests and ultrasound-screening of both partners, albeit selectively for the male. Male examination includes the measure of testicular volumes by Prader orchidometry, as well as the detection and grading of varicoceles.
- Fertility clinicians should embrace both Gynecology and Andrology in a sub-specialty classification which ensures appropriate training.
- Fertility treatments must be integrated for both Andrological and Gynecological perspectives at every level including clinical, laboratory, ultrasound, and genetic assessments. Ideally the data from these areas is collected real-time into a single data-base for subsequent audits and evaluations.
- Those men treated for varicoceles need appropriate surgery meaning microsurgical ligation of the testicular veins in facilities well integrated with the Fertility Centre which ensures no delay in fertility management from the female perspective.
- Fertility Centres, including the integrated ancillary services such as Surgical Andrology must embrace audit and critical evaluation in an accreditation process, preferably subject to annual review.

The role of sperm DNA fragmentation (SDF) screening

Against this historical background, the readers of this journal are favourably encouraged to absorb the detail

of the article in this issue from Laboratory Andrologist Ashok Agarwal (PhD) of the esteemed Cleveland Clinic in Ohio, USA and his Surgical Andrologist colleagues (Board certified Urologists) from other equally esteemed Clinics in the USA, Brazil and Canada (16). These surgical-oriented colleagues have embraced Andrological issues over the past three decades and shown the way in managing the male aspects in the setting of infertility. Their article on the clinical utility of SDF testing provides practice recommendations based on actual, and typical clinical scenarios. Their presentation fully describes the current SDF tests with the methodology and principles of action, along with comparative advantages and disadvantages of each method. They rightly promote the application of SDF testing as a routine. Although there are several described limitations, the results indicate lower biological variability than conventional semen analysis.

These authors, as Andrologist and Urologists, are providing helpful advice to Fertility Clinics facilitating ART services, and which are essentially staffed by gynecologists as the primary assessors of infertility cases, but who will need to consider what criteria governs the need for a urological referral. Agarwal and his co-authors present several clinical scenarios where the semen analysis profile ranges from normal or unexplained infertility, to cases with both mild and severe oligoasthenoteratozoospermia. Each of the four case-scenarios presented had management outcomes which were likely influenced by SDF assessment, including the case of miscarriage at 10 weeks gestation of an ICSI pregnancy.

Responding viewpoints

We have two views to present about this important study.

Firstly, the case scenarios are all highly relevant for SDF testing, namely:

- Cases with clinical varicocele—high DNA fragmentation may be the best guide for ligation.
- Cases with unexplained infertility, recurrent pregnancy loss and IUI failures—high DNA fragmentation may be the underlying problem.
- Cases with repeated IVF or IVF/ICSI failures—high SDF may indicate the need for using sperm derived from the testis, rather than the ejaculate, to avoid prolonged ROS exposure within the epididymis.
- Cases with clear lifestyle factors e.g., smoking (17), obesity, occupational toxins and organochlorine pollutants—high SDF can point to the need for lifestyle modification and use of anti-oxidants (18).

In all these scenarios, the raised DNA fragmentation levels are sheeted home to the likely detrimental influence of excessive amounts of ROS (19), although the precise mechanism of ROS action remains unclear at this stage. With respect to bypassing the epididymis, more research is required as this organ is important for optimal sperm maturation (20), but can be severely damaged in cases with long-term obstruction of the vas (21). Nonetheless, our current practice is to search for debris-free micro-epididymal sperm aspiration (MESA) samples containing motile spermatozoa in preference to testicular sperm extraction (TESE) samples, to achieve optimal live-birth rates (22).

Our second view concerns the structure of ART Centres and their individual policies regarding the referral of cases for specific further specialist management including Endocrinological, Medical Andrology, Surgical Andrology (Urological Surgeon), Genetic Counselling, Gynaecological surgery, etc. In some countries, there are territorial boundaries so that only Urological Surgeons can undertake operations such as varicocele ligation whereas in others, such as Australia, such boundaries are not strict and fertility specialists with higher sub-specialist qualification such as certification in reproductive endocrinology and infertility (CREI), may undertake procedures on the male without a specific surgical qualification in Urology. The majority of MESA and TESE cases are undertaken by Gynecologists or Physicians with internal training, and not by Urologists, albeit many apply a sub-optimal needle aspiration TESA method, which requires repetitive procedures for many cases. Some clinics have tried to integrate Urologists into the ART Centre, but such applies to only a couple from a total of more than 70 facilities. At this stage, referral to a Urologist occurs only when a testicular tumour is suspected (23), usually incidental in those clinics who utilize routine ultrasound of the genitourinary system of the male (6). Urologists in Australia have not complained about this as they appear to be very busy dealing with renal, prostate and bladder problems outside the scope of infertility and only two Urologists to my knowledge, have committed to Andrology—one in Sydney, another in Melbourne.

Integrating Surgical Andrology

Nonetheless, it would appear that the lack of involvement of Surgical Andrologists in ART Centres is a significant failing, highlighted recently by a commentary from the

Department of Urology at Baylor College in Texas (24). They commentated on opportunities lost for men with varicoceles; many of whom, with either azoospermia or oligozoospermia would have benefited by appropriate ligation surgery prior to ART procedures (25). The suggestion by Agarwal and his co-authors in Surgical Andrology advising the use of SDF screening in order to decide which males should be referred is an excellent proposal, albeit that the cut-off level may be unclear at this stage; perhaps 15% DNA fragmentation, rather than 30%, might be considered as cases of completely unexpected total fertilization failure can occur in IVF above this level (26).

Future directions in Laboratory Andrology

However, the idea of a single laboratory test like SDF to decide which men require closer attention (such as referral to a urologist), remains problematic in the sense of oversimplification and supportive of the current inadequate approach to the male (excepting in those few highly integrated Fertility Centres). Furthermore, in our Australian facility, we have been disappointed in the inability of SDF screening to detect all cases requiring ICSI, hence we undertake a split IVF-ICSI model on all new cases to determine the optimum model if future treatments are required (26). Another consideration is that of sperm selection for the generation of embryos of optimal quality and minimized aneuploidy rates; SDF testing points to those cases where the sperm selected for ICSI will require an advanced detection method—future research yet to be reported.

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Footnote

Conflicts of Interest: The authors have no conflicts of interest to declare.

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