Sperm functional tests

Sergio Oehninger, M.D., Ph.D.,[a] Daniel R. Franken, Ph.D.,[b] and Willem Ombelet, M.D., Ph.D.[c,d]

[a] Department of Obstetrics and Gynaecology, Jones Institute for Reproductive Medicine, Eastern Virginia Medical School, Norfolk, Virginia; [b] Department of Obstetrics and Gynecology, University of Free State, Bloemfontein, South Africa; [c] Genk Institute for Fertility Technology, ZOL Hospitals, Genk; and [d] Faculty of Medicine and Life Sciences, Hasselt University, Hasselt, Belgium

Several semen parameters are used to discriminate the fertile male from the subfertile male. The most widely used parameters are sperm concentration, motility, progressive motility, and sperm morphology. Semen analysis is usually applied as described in the World Health Organization manual for semen analysis. In addition to a routine semen analysis, sperm functional tests have been described for many years, which in most cases are regarded as research tools and not part of the routine semen testing in an infertility clinic. In this review we report on the value of four sperm function tests: the sperm penetration assay, the sperm–zona pellucida binding tests, the acrosome reaction, and the hyaluronan binding assay. For each test we describe the current value, the indication for performing the test, how to interpret the results, and its therapeutic implications. Our data show that sperm functional assays are highly predictive of IVF outcome results and have the potential to assist in clinical decision making, especially to avoid the current long-standing treatment with IUI and to direct the patients to intracytoplasmic sperm injection without delay when sperm functional testing fails. We believe that advances in molecular biology techniques will allow us to develop simpler sperm function assays in the near future. This will undoubtedly help clinicians in optimizing male factor infertility diagnosis and treatment. (Fertil Steril® 2014;102: 1528–33. ©2014 by American Society for Reproductive Medicine.)

Key Words: Acrosome reaction, male infertility, sperm functional test, sperm penetration assay, sperm–zona pellucida binding tests

Discuss: You can discuss this article with its authors and with other ASRM members at http://fertstertforum.com/oehningers-sperm-functional-tests/

Use your smartphone to scan this QR code and connect to the discussion forum for this article now.*

* Download a free QR code scanner by searching for "QR scanner" in your smartphone's app store or app marketplace.

The basic semen analysis has limited predictive value for pregnancy in couples trying to achieve natural conception and in couples undergoing advanced assisted reproductive technologies (ART) (1). This highlights the need for more extended sperm functional testing. Ideally, the sequential analysis of sperm functions could assist clinicians in planning the therapeutic approach and predicting the outcomes of such treatments (2–8).

In the last two decades the intracytoplasmic sperm injection (ICSI) setting has provided a new and unique arena to evaluate sperm dysfunction. After the first successful ICSI deliveries (9, 10), the clinical focus immediately shifted to gamete manipulation. ICSI quickly became the selected technique for cases of male factor infertility and for couples with previously failed fertilization with conventional IVF. Furthermore, it was demonstrated that the basic semen parameters of the unprocessed ejaculate or even after separation of the fraction with highest motility had no impact on the outcome of ICSI (11, 12). This was followed by achievement of high levels of fertilization with ICSI in the presence of multiple morphological and dysfunctional sperm defects, as well as after the use of ejaculated testicular or epididymal sperm or cryopreserved-thawed sperm and, in cases of obstructive and nonobstructive azoospermia, after sperm extraction from testis or epididymis (13).

In spite of the fact that ICSI has remarkably improved male factor infertility results in ART, we continue to face daily clinical dilemmas. The answer to the many current challenging questions relies on the unveiling of spermatogenesis pathologies and the resulting sperm dysfunctions at the cellular and molecular levels. The role of the various spermatozoal components suspected of actively participating in early human development has being reevaluated (14, 15). The contributions of the fertilizing spermatozoon to the oocyte include, as a minimum, the delivery of the DNA, a putative oocyte-activating factor, most likely phospholipase C zeta (16), and a centriole. Although irrefutable evidence is needed, phospholipase C zeta is now widely considered to be the physiological agent responsible for activating mammalian oocytes (17, 18). It has been also established that the fertilizing spermatozoon may also provide the zygote with a unique suite of paternal mRNAs (19) and that some transcripts might be crucial for early and late embryonic development (20). Clinicians are still looking for the elusive functional test that could be applied universally at the laboratory.

Received August 2, 2014; revised September 27, 2014; accepted September 30, 2014; published online October 24, 2014.
S.O. has nothing to disclose. D.R.F. has nothing to disclose. W.O. has nothing to disclose.
Reprint requests: Willem Ombelet, M.D., Ph.D., Department of Obstetrics and Gynecology, Genk Institute for Fertility Technology, ZOL Hospitals, Schiepse Bos 6, 3600 Genk, Belgium (E-mail: willem.ombelet@telenet.be).

Fertility and Sterility® Vol. 102, No. 6, December 2014 0015-0282/$36.00

http://dx.doi.org/10.1016/j.fertnstert.2014.09.044

Work derived from the early IVF days demonstrated that defective acrosome reaction and/or abnormal sperm–zona pellucida (ZP) interaction was frequently observed in the ejaculated sperm of infertile men. Such findings were observed in the presence of normal or abnormal "basic" sperm parameters. Both types of dysfunctions were shown to result in fertilization failure or low fertilization rates. Consequently, acrosome reaction tests and sperm-ZP binding assays were developed to address a real need to assess sperm functional competence in the "extended" evaluation of the infertility workup before conventional IVF was performed. In the current ICSI era, the results of such functional assays can still provide valuable information to the clinician so that he or she may recommend against low complexity alternatives such as IUI therapy and direct couples to ICSI (6, 8, 21–25).

The World Health Organization (26) qualifies sperm functional assays as research tests. These tests were originally conceived as tests to predict the fertilization potential of the male gamete in vitro. Nonetheless, their power to predict pregnancy, a multifactorial outcome, has also become more evident. These bioassays include the examination of sperm binding to the ZP, acrosomal exocytosis, and fusion with the vitelline membrane of the oocyte. The binding of spermatozoa to the ZP initiates the acrosome reaction, releases free and exposes bound lytic acrosomal components, and allows the spermatozoa to penetrate through the zona matrix, driven by the increased flagellar thrusting of hyperactivated motility (27). Although the results of some of these assays correlate with fertilization in vitro with high statistical significance, there are definite drawbacks to their performance, including the need for human material (i.e., ZPs to be solubilized and/or intact eggs), and they are technically and time demanding, making them awkward in the routine clinical laboratory (21).

One of the few published meta-analyses on sperm-oocyte interaction assays revealed that the sperm–ZP binding assays, that is, a sperm-zona binding assay and the hemizona assay (HZA) (28, 29), and acrosome reaction tests, including the examination of ZP-induced acrosome reaction (30, 31), provided clinically useful and prognostic information related to sperm competence to fertilize mature eggs in the IVF setting (22, 24, 32). The HZA also proved to be a predictor of IUI outcome in couples with male factor infertility (33). Within the IVF setting, it was established that the extremely high and frequently observed morphological abnormalities of the male gamete (teratozoospermia) could be used as a biomarker of several gamete dysfunctions, including dyskinetic disorders, and altered capacity to interact with the egg and its vestments (5, 6, 22, 23, 34, 35).

To fertilize the egg, ejaculated spermatozoa must undergo capacitation, recognize and bind to the ZP, and undergo the acrosome reaction. The most significant changes experienced by sperm during capacitation are plasma membrane changes, an increase in certain intracellular messengers, and increased phosphorylation of a set of proteins by different kinases (36–38). Capacitation was first observed in the rat when sperm injected into the periovarian sac of the rat after ovulation did not begin to enter the eggs until 4 or 5 hours later (39, 40). Similar findings were reported in the rabbit when sperm were able to fertilize more eggs if they had first spent about 5 hours in the uterus of another rabbit (41). Sperm capacitation studies require the use of an in vitro fertilizing system. This phenomenon was initially accomplished using cauda epididymal sperm and/or ejaculated sperm incubated under a variety of conditions in defined media mimicking the electrolyte composition of the oviduct fluid.

The molecular and physiological events that enable sperm to fertilize in the female tract are collectively known as capacitation (38, 42–44). The actual capacitation process can be monitored using an antibiotic chlortetracycline that yields different patterns of molecules distribution on the sperm surface that can be visualized as distinct fluorescence patterns depending on the capacitation and the acrosomal status of the sperm (45).

Assessing the ability of human spermatozoa to acquire fertilizing potential (capacitation) by stimulating exocytosis of the contents of the acrosome (acrosome reaction) is thought to have diagnostic potential (44). Calcium-mobilizing agents, such as calcium ionophores (A23187) and P, stimulate the acrosome reaction in vitro (46). Acrosomal status is easily detected using the lectin Pisum Sativum Agglutinin labeled with fluorescein isothiocyanate (47). Defective calcium influx and acrosome reaction (spontaneous and P induced) were found to be compromised in the spermatozoa of infertile men with severe teratozoospermia (48), providing further evidence for the use of capacitation endpoints (acrosome reaction and sperm–ZP binding) as male diagnostic tools.

The aim of this review was to review and highlight the clinical value of sperm functional assays.

SPERM PENETRATION ASSAY (SPA)

This test was one of the first bioassays of sperm function developed (49–51). In this heterologous system, human sperm were subjected to capacitating conditions and incubated with hamster oocytes devoid (enzymatically) of the ZP. The sperm penetration assay with zona-free hamster ova was widely used in the pre-ICSI days. The SPA measures the spermatozoa's ability to undergo capacitation, acrosome reaction, fusion and penetration through the oolemma, and decondensation within the cytoplasm of hamster oocytes and was used to evaluate male fertility potential. However, the results have remained difficult to interpret.

Mao et al. (52) evaluated the clinical relevance of the SPA and reported sensitivity ranges from 0.00 to 1.00 and specificity ranges from 0.95 to 1.00 for diagnosing male factor infertility. As a prognosticator of IVF failure, the sensitivity varied from 0.00 to 0.78 and specificity ranged from 0.51 to 1.00. Similar reports indicated by Vogiatzi et al. (53) reported considerable variation in the diagnostic accuracy values of SPA with wide sensitivity (52%–100%), specificity (0–100%), and positive predictive value (PPV; 18%–100%) and negative predictive value (NPV; 0–100%) together with fluctuation and notable differentiation in the methodology and cutoff values employed by each group. The reproducibility of this assay and standardization of methods between laboratories was low.

The conventional SPA depends on the occurrence of spontaneous acrosome reactions in populations of spermatozoa incubated for prolonged periods in vitro. The fusion of