Editorial Articles

The infertile male - Diagnosis

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Summary

Purpose: To discuss a variety of tests used to diagnose the subfertile male and to impart based on clinical experience, reading, and personal research, this editor's view of the relative value of these tests.

Methods: The tests discussed include motile density, sperm morphology, the hypo-osmotic swelling test, antisperm antibodies, sperm chromatin structure assay, DNA integrity tests, reactive oxygen species, sperm penetration assay, sperm-zona pellucida binding tests, sperm creatine kinase activity, plasma membrane mannose-ligand receptor assay, and nuclear morphology.

Results: Except when extremely low (< 2.5 x 10^6/ml) motile density does not identify the subfertile male very well. In contrast to other studies, my group's data suggest that neither low normal morphology by WHO standards or strict criteria identify the subfertile male. The best predictor of male subfertility is the hypo-osmotic swelling test when it is < 50%, which does not result in fertilization failure, but implantation failure. A high percentage of sperm coated by antisperm antibodies is very predictive of fertilization failure.

Conclusions: The physician must be careful when concluding that the male is subfertile or fertile based on standard tests of concentration, motility, and especially morphology.

Key words: Semen analysis; Morphology; Motile density; Implantation defects.

Introduction

Diagnosing male factor as a cause of infertility

The first question is since my official position is a Professor of Obstetrics and Gynecology at Robert Wood Johnson Medical School, Camden, NJ and the Head of the Division of Reproductive Endocrinology and Infertility am I really qualified to discuss evaluation and treatment of the infertile male? Traditionally, the male is evaluated by a urologist. There are some urologists that specialize in male infertility.

However, I believe it is easier to treat the couple as a whole, and it is my opinion that the male should be evaluated and treated by a reproductive endocrinologist and the role of the urologist should be for surgical procedures, e.g., sperm aspiration from the testes. I certainly do not think I am nearly competent enough to examine a male and, for example, detect a small varicocele. However, since my opinion is that the very large majority of males with a varicocele should not have a varicocelectomy, to me it does not matter if the male partner has one or not. Interestingly, I went to a one-day male infertility post-graduate course at the 2005 American Society for Reproductive Medicine that was conducted by three urologists and the chairman made the statement that males with prolactinomas should be treated by urologists rather than endocrinologists because in his opinion endocrinologists mostly treat diabetes! Thus, I do not feel so bad making my statement.

Do I qualify to at least give an opinion (right or wrong) based on the tenets that I set forth previously in the editorial "The Diagnosis and Treatment of Infertility – One Person's Philosophic Approach"? I have been scientifically evaluating male factor for almost 30 years as evidenced by my first publication concerning male infertility published in *Fertility and Sterility* in 1977 dealing with the treatment of males with low sperm count and motility with the drug clomiphene citrate [1]. My group has performed semen analyses ourselves for over 30 years and I have evaluated male factor by serum hormonal levels since that time. In these 30 years I have written over 125 peer review manuscripts dealing with male factor and these publications include recent publications in 2004 and 2005 including 18 manuscripts. Some manuscripts have been accepted for publication and will be published shortly in journals. In the last 12 months three of the nine research presentations at the 2005 American Society for Reproductive Medicine (ASRM) involved the infertile male, all five presentations at the American Society of Andrology, and two of nine presentations at the 2005 ASRM meeting. Though I did not attend the World Congress of Andrology meeting in Korea in June, I prepared the nine presentations (three of my staff presented the data). However, I did have the benefit of reviewing the literature to prepare these presentations and receive feedback from the meeting. Furthermore, immediately before writing this editorial not only did I attend a one-day post-graduate course on male infertility, but I attended two research sessions during the meeting and read many posters dealing with the infertile male. We have four more presentations for the American Society of Andrology coming up in the spring of 2006 and the posters are now being prepared.

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Part I - Diagnosis of the infertile male

One of the first tests that should be performed on an infertile couple is the semen analysis. The standard semen analysis includes measuring the sperm volume, the concentration of sperm (million/ml), the total sperm count (concentration multiplied by volume), the percentage of sperm that are motile, the quality of motility (fast and straight, moderate speed with somewhat circuitous motion, slow or moving in place), motile density (concentration of sperm/ml multiplied by % moving), concentration of white blood cells, and morphology (2 types - one by the World Health Organization (WHO) standpoint or another called Kruger's strict morphology). There are other important things noted also, e.g., ability to liquefy, color, etc.

In some instances, e.g., for religious or personal reasons, the male partner cannot produce a semen sample. In this case, a postcoital test can be substituted where two to three days before actual ovulation the mucus from the cervix is gently extracted and evaluated for the presence of motile sperm from intercourse the night before. The presence of motile sperm at least assures that the volume is sufficient to find the cervical os and the sperm concentration and quality of motility is sufficient, and "stamina" of the sperm is sufficient to remain motile at least eight hours after intercourse. Furthermore, if a normal postcoital test occurs, then one can generally assume that the male partner does not have clinically significant antisperm antibodies (there are exceptions which will be discussed later).

Sperm concentration and motility

When I first started practicing infertility over 31 years ago a normal sperm concentration was considered to be 100 x 10^6/ml and normal motility was 60%. Thus a motile density of 60 x 10^6/ml was considered normal. By 1992 this number had been reduced to 40 x 10^6/ml and 20 x 10^6/ml as low-normal. With 50% motility considered normal then by the WHO a normal motile density was considered to be 10 x 10^6/ml. Thus 25 years ago if a man had varicocele and had a motile density of 32 x 10^6/ml a varicocelectomy would have been suggested. By 1992 it could be suggested that surgery be performed for low normal cases (i.e., $\geq$ 10 x10^6/ml but $\leq$ 20 x 10^6/ml) but for the most part surgery would be considered if the motile density was < 10 x 10^6/ml.

We performed a research study in 1991 to determine if this new low cutoff defined subfertility [1]. For infertile couples where a correctable female infertility factor was identified the couples in the study were asked to merely have intercourse without treatment of the male and only the female problems were corrected. Pregnancy rates were determined according to the motile density of the male partner (Table 1). Eighty-one percent of the couples where the motile density for the male partner was superior (> 20 x 10^6/ml) achieved a pregnancy in six months but so did the group with subnormal motile density at 5 x 10^6/ml to 10 x 10^6/ml motile sperm. Even couples with male partners' motile densities at 2.5 to < 5 x 10^6/ml had a respectable six-month pregnancy rate of 69%. There were still pregnancies, albeit considerably reduced (22%), for those < 2.5 x 10^6/ml. Today 40% motility is considered normal and a subnormal motile density is < 8 x 10^6/ml.

Table 1. — *Correlation of motile density (MD) and pregnancy rates (PRs) during a 6-month interval.*

	MD (mil/ml)				
	< 2.5	≥ 2.5-< 5	≥ 5-< 10	≥ 10-< 15	≥ 15
# couples	32	13	31	34	171
# pregnant	7	9	25	27	139
% pregnant	22	69*	81*	79*	81*

*p < .001 compared to group 1.

I do not have a study to prove it but from a clinical observation I think that a male with a subnormal motile density of 5 x 10^6/ml who has a low sperm concentration of 10 x 10^6/ml but 50% motility is more fertile than a male with 50 x 10^6/ml sperm concentration but only 10% motility. The quality of the motility is also important; certainly it is better to have more sperm with rapid progressive linear motion than poorly motile non-progressive sperm or sperm just moving in place.

When one is not sure about sperm quality, it is always reassuring to find them moving in the mucus many hours after intercourse. It is interesting that many years ago a meta-analysis concluded that it is not "cost effective" to do a postcoital test [2]. A postcoital test only needs to be done once (unless the female partner begins clomiphene citrate) [3], is painless, risk-free, and should not cost more than the office visit. Thus this seems to be a ridiculous statement [4]. Yet I notice that a lot of patients seeing infertility specialists have never had one performed. Many are having instead very expensive intrauterine insemination requiring hours lost from work whether the couple needs it or not. One of the problems with analyzing previous data with this test is that it is not always clear that the test was performed at the right time, i.e., when the serum estradiol level is high enough but before the rise in the LH surge [5]. If the postcoital test is performed at the proper time and the mucus quality is poor and the sperm volume is normal, and there are no antisperm antibodies attached to the sperm (antisperm antibodies in cervical mucus is rare) [6], but no or rare sperm are seen, this informs me that the male partner needs treatment or that certain procedures need to be performed on the female partner such as intrauterine insemination (IUI) [7] or in vitro fertilization (IVF).

It should be kept in mind that low concentration or poor motility of sperm may be temporary and a much improved specimen may be found at a subsequent date without any treatment. Sometimes certain conditions, e.g., fever, may cause a temporary problem and a much improved specimen may be found at a subsequent date without any treatment. Sometimes no definite cause is known but the sperm specimen suddenly improves.

Sperm morphology (shape of the various organelles making up the sperm)

The WHO established a set of criteria but these criteria did not seem to identify the subnormal male [8].

However, a new set of criteria were established by Kruger *et al.* and these were much stricter [9]. The claim was made that when a male had < 4% normal sperm using strict criteria pregnancy with intercourse, IUI or conventional IVF (intracytoplasmic sperm injection [ICSI] had not been introduced as yet) was not possible or extremely rare [10, 11].

Workshops from around the world were set up to teach various infertility centers to perform this new assay. The creators of this technique would show a world map of all the centers around the world that confirmed their data. There was, in fact, only one infertility center that did not agree and that was referring to our publication in 1992 [12]. In that study, without IVF or IUI, we did find a lower pregnancy rate with males with normal motile density and low morphology but a 40% six-month pregnancy rate certainly was not consistent with claims that his is one test when abnormal predicts no or rare pregnancies [12]. Interestingly, however, not only did males with low motile densities and low morphology achieve pregnancies, but the highest pregnancy rates were found with the men with strict morphology < 4% [12].

Subsequently using conventional IVF (50,000 sperm incubated with the egg) and not intracytoplasmic sperm injection where one sperm is injected per egg, we found that when the sperm had low motile density as the only problem there was no difference in pregnancy rates compared to males with all semen factors normal [13]. However, the males with strict morphology of ≤ 4% achieved a pregnancy rate almost twice as high as these two other groups. The group with a low hypoosmotic swelling test score had a zero percent pregnancy rate (I will be discussing this test later).

A scientist, renowned for various meta-analyses, gave a keynote speech at the Pacific Coast Reproductive Society Meeting and concluded that evaluating sperm using strict criteria is a very valuable test. At the World Congress of IVF another well known infertility specialist in a keynote speech stated that the best test to detect a subfertile male is strict morphology and he even upgraded the level to include ≤ 5% normal. Also recently one of my associates attended a board review coarse for reproductive endocrinology and infertility and one of the assigned teachers also stated that strict morphology is the best test to detect the subfertile male. Nevertheless, at a male fertility post-graduate course at the American Society for Reproductive Medicine meeting in October 2005, the consensus was that strict morphology has limited diagnostic potential. In fact we reevaluated the importance of strict morphology in 2002 and found that the pregnancy rate/cycle with IUI was 30% with strict normal morphology at 0-4% vs 26% for 5-14% vs 20% for > 14% [14].

All I can say is that based on my own data I do not agree that low normal morphology by strict criteria predicts male subfertility and I certainly would not jump into IVF with ICSI based on poor morphology, especially if the expense is coming out of the couple's pocket. I would emphasize looking for subtle abnormalities in the female partner and at most, in the beginning, might consider timed IUI (though I do not know if even that is necessary). Though I would definitely consider IVF if there has been many cycles of failure (a woman ≤ 35 who has failed 8 cycles of therapy probably only has a success rate of 15-20% for the next 8 cycles if the same therapy is continued). However, I would consider IVF in this case even if the semen was perfectly normal.

Antisperm antibodies

I think most infertility specialists agree that antisperm antibodies, where a male produces immunological proteins directed against certain components of the sperm, reduce sperm fertility potential. The most commonly used test is the immunobead test where a bead is attached to anti-human gammaglobulin. If an antibody (which is a human gammaglobulin) is present it will attach to the sperm, and the bead will be visible. One can then determine what percentage of sperm have any antibodies attached, but it does not detect how laden with antibodies is a given sperm. Mild levels (< 50%) are not usually associated with much if any reduced fertility. The closer the level is to 100%, the worse the fertility prognosis.

Antisperm antibodies prevent conception in two main ways: either by immobilizing the sperm in the cervical mucus preventing them from access to the fallopian tubes or by inhibiting attachment of the sperm to the zona pellucida.

The presence of antisperm antibodies does not usually immobilize the sperm in the semen container [15]. The reason for this is that for the antisperm antibody process to be complete and cause immobilization a third component is needed, i.e., complement. A normal male, even one with antisperm antibodies, does not have complement in the ejaculate; thus the motility may seem perfectly normal [15]. However, there is complement in the cervical mucus and the sperm thus become immobilized.

It takes several hours for the sperm antigen, antibody, and complement reaction to occur. Thus a couple may show a normal postcoital test two hours after intercourse but at eight hours no sperm are moving any longer. Thus the postcoital test can be a screen for antisperm antibodies but it is important to evaluate at least eight hours after intercourse due to the delayed effect of the antigen/antibody/complement reaction.

One question that arises is whether it is of any value to measure antisperm antibodies if the postcoital test is normal. Indeed we have demonstrated a much better prognosis for males with antisperm antibodies if the postcoital test is normal [16]. However, though most males with antibodies blocking attachment to the zona pellucida also have immobilizing antibodies, in some refractory cases of infertility, the only antibodies present are those preventing fertilization

by blocking attachment to the zona pellucida. Therefore, it is my preference to evaluate antisperm antibodies on the initial semen analysis. However, I could see the argument that to be cost effective why not perform the test only in those couples with abnormal postcoital tests or those failing to conceive in six or eight months after seemingly correcting all infertility factors. Strategies for treatment will be discussed later.

The hypoosmotic swelling test

The hypoosmotic swelling test (HOST) can detect an interesting abnormality. It is generally assumed that the purpose of having an adequate volume and concentration of sperm with adequate motility, and the absence of having antisperm antibodies is to allow enough sperm to travel through the cervix into the uterus, then the fallopian tubes to reach the egg in the upper third of the fallopian tube, and then have enough energy left to allow 300 or so sperm to attach to the zona pellucida thus allowing one sperm to penetrate and fertilize the egg. The classical concept is that once fertilization occurs the job of the sperm is finished. Thus it is assumed that as long as the egg is fertilized the sperm can no longer be considered as a contributing cause to persistent infertility. This is not a true assumption and is a mistake made by not just some, but in my opinion, the majority of infertility specialists.

We first published data in 1989 demonstrating that males with normal semen parameters but HOST scores < 50% do not achieve pregnancies following intercourse [17]. However, several subsequent research publications by other authors stated that a low HOST score had no adverse effect on fertilization rates following IVF-ET [18-21]. Thus, most infertility specialists assumed that this means that a low HOST score has no importance.

What is interesting, however, is that not one of the groups publishing these data on fertilization rates presented their pregnancy rates [18-21]. So we performed our own matched-controlled study performing IVF with conventional oocyte fertilization and found, similar to these other authors, no adverse effect of low HOST scores on fertilization rates. However, there were hardly any live pregnancies [22]! The aforementioned study evaluating single-sperm defects on IVF outcome found a 25% clinical pregnancy rate/transfer with all semen parameters normal and with low motile density, 44% with low strict morphology but 0% with a HOST score < 50% [13]. A study where a single pool of oocytes was shared between two male partners with normal semen parameters but one with a normal and the other a subnormal HOST score was performed. The clinical pregnancy rate/transfer was 50% in the former vs 0% in the latter [23]. Despite these dramatic findings, and the inexpense and simplicity of the test, my experience from consulting with many infertile couples who have seen previous specialists is that most treating physicians do not order this very important test. Thus I believe that most infertility specialists are not aware that some sperm pathologies can allow fertilization of the egg but can prevent the embryo from implanting [24].

The 50% cutoff is critical for this test. Jeyendran *et al.* stated that the grey zone for this test was 50-59% [25]. However, we did not find any reduced pregnancy rates with male partners with grey-zone HOST scores [26]. We have found that the HOST abnormality once it is subnormal generally stays subnormal [27]. A frequency of this abnormality was found in 8% of the male partners aged < 45 in our infertile population, 16% in males aged 45-49, and 33% in males ≥ aged 50 [28].

The basis of this single test is as follows. There is a higher concentration of water in the hypo-osmolar medium than inside the sperm. When there are two concentrations of water across a membrane, water will be pumped by the membrane from the high to the low side to attain equilibrium through active transport. If the sperm membrane is functionally intact it will pump the water from the hypo-osmolar medium to the inside of the sperm, thus causing the sperm tail to swell. Thus the test is inexpensive, easy to measure, and very reproducible. Normal males should have ≥ 50% of the tails exhibiting swelling when exposed to hypo-osmolar media.

It could be argued that if swelling indicates normal sperm what difference does it make if a man has a HOST score of 40% but a high sperm concentration and good motility? Are there not enough good sperm present? I will present evidence during the treatment section that this defect is more of a toxicity issue, i.e., there is a toxic protein attached to the sperm that causes this defect. This toxic protein is transferred to the zona pellucida by the supernumerary sperm with the toxic factor attaching to the zona pellucida. The egg membrane becomes incorporated in the embryo membrane and thus this toxic protein is transferred to the embryo membrane. The hypothesis continues that the presence of the toxic protein in the embryo membrane impairs the functional integrity of the embryo membrane. This impairs proper attachment of the embryo to the endometrium or prevents invasion of the trophoblast into the endometrium [24]. I will present the evidence supporting this hypothesis when the treatment of this defect is discussed.

Sperm chromatin structure assay (SCSA)

The SCSA test detects the ability of the sperm chromatin to resist acid denaturation by measuring DNA fragmentation [29]. Thus the assumption is that the main nuclear material that is responsible for fertilization is defective. Similar to the low HOST, some data showed fertilization rates with IVF were normal, but the embryos did not implant when fertilized with males who had high DNA fragmentation indices (DFI) of > 30%, at least when conventional insemination of oocytes was used or ICSI [30]. However, subsequent data did find pregnancies with high DFI scores of > 30% and in fact found the main effect to be the cause of an increased miscarriage rate [31, 32]. Recently some studies have questioned whether this test has any value at all in predicting outcome, at least following IVF-ET [33-35].

Other DNA integrity tests

The SCSA test is an indirect measurement of DNA integrity. There are suggestions that tests that directly measure DNA damage, e.g., the TUNEL, in situ nick translation or COMET assays may better predict abnormal sperm than the SCSA test [36]. We usually offer the option to male partners to have the SCSA test performed on their initial semen analysis which includes also measurement for antisperm antibodies and HOST, but most opt to do it at a later time if they are not successful in achieving a pregnancy during some time interval to save money since the SCSA test is not covered by insurance.

Other tests of sperm function

Reactive oxygen species

There are several other tests that are not commonly used but may have merit in detecting the subfertile male, e.g., reactive oxygen species or oxidants [37-40]. The sperm stress test may also predict sperm that can lead to normal fertilization rates but poor implantation rates but is rarely used [41]. I am not sure if Alvarez and co-workers feel as strongly about the test as they did before [41].

Sperm penetration assay

The zona-free hamster egg penetration assay (SPA) evaluates sperm-egg fusion. Oehninger *et al.* evaluated 12 IVF studies including 842 subjects. There was a positive predictive value of over 70% but lower specificity and high false-negative rates [42]. There have been variable conclusions for in vivo studies. One study using a 15% cutoff showed that many women conceived despite an SPA < 15% [43]. The test is expensive, difficult to perform, and may vary from specimen to specimen. Probably it should be restricted to couples failing to conceive after many seemingly perfect cycles and even then the results should be given only limited credence.

Sperm zona pellucida binding (SZB)

There are two main types of SZB assays. The SZB test uses oocytes that fail to fertilize in vitro [44]. The meta-analysis by Oehninger *et al.* [42] included ten studies, of which seven used the hemi-zona assay and three used the sperm-zona binding test. Eight studies could be combined and the positive predictive values were ≥ 80% (range 79-95%) and the negative predictive values were generally > 70%.

Sperm creatine kinase activity

Creatine kinase is the key enzyme in the synthesis and transport of energy. Creatine kinase levels are used to distinguish fertile from subfertile males with oligozoospermia; higher levels are found in subfertile specimens and signify defective spermatogenesis and thus subsequent lower fertilization rates [45, 46]. Improved accuracy was obtained by determining the ratio of the presence of a specific isotype of creatine kinase muscle-type (M-type) to the total amount of creatine kinase present (M-type plus brain (B) type) [47]. Men were classified as CK-MM infertile with a ratio of < 10%, and no pregnancies following IVF-ET occurred compared to 23% in those with ratios > 10% [47]. The automated technology for CK isoform measurements is readily available. Furthermore, the CK-MM ratio evaluates sperm development rather than selected functions, e.g., motility or acrosomal status.

Plasma membrane mannose-ligand receptor (PMMLR)

Normozoospermic men who fail to fertilize oocytes following IVF have a defect in the ability to increase the percentage of sperm with plasma membrane mannose-ligand receptor expression over the acrosome and postacrosomal regions of the sperm head, and the percentage of sperm exhibiting spontaneous and mannose-induced acrosome reactions following sperm incubation under standard capacitating conditions [48, 49]. Most men have normal values, which only makes it valuable for a small minority of patients (personal experience). A high frequency of abnormalities in mannose-ligand receptor expression has been noted in men taking calcium-channel blockers [50]. However, a subsequent study failed to find any association of men taking calcium channel blockers and male subfertility [51].

Nuclear morphology

Support of our conclusions about the lack of great value in using strict morphology to predict male subfertility was provided by demonstrating with high power magnification (6500x vs 400x normally used to evaluate morphology) that not one organelle (head, tail, neck piece, acrosome, etc.) abnormality that would cause a sperm to be considered abnormal when determining strict morphology correlated with pregnancy outcome [52]. However the study did find that abnormal nuclear shape or the presence of nuclear vacuoles did correlate with poor pregnancy outcome [52]. Nuclear detail cannot be seen and thus is not included when determining strict morphology which is evaluated at 400x.

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