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Review Article

Infertility-Research Methodology and Applied Aspect of Semen Analysis

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Abstract:
Many Ayurveda colleges of the country are running Vajikarana clinics and maintaining semen analysis laboratory, where male infertility is being treated as well as research is being done to assess the role of Ayurveda drugs for itsmanagement. For the facilitation their research work as well as for better interpretation of the findings it is necessary to have a detailed knowledge of the applied aspect of the various tests being performed for the semen examination. For the facilitation of their research works in this paper various methods of semen examination, their normal and abnormal values and clinical implications etc. are described. Some new and latest development took place in the field are also discussed.
Key Words: Semen, semen analysis methods and interpretation, male infertility

Introduction:

Prof Gurdipsingh has been of the opinion that Ayurveda research scholars as long as possible perform their research work by themselves and for that purpose the necessary laboratory and instrumental facilities should be made available in the institute/work place itself. In view of this he established a research Laboratory in Kayachikitsa Department Jamnagar in 1978 where Rasayana drugs were screened for their immunity promotion effects which opened a new avenue for the Rasayana drugs. Later on in 1993 under his guidance Dr. B. Sreenivasa Prasad established semen analysis laboratory with the technical help of Dr. K.P. Skandan, then Associate Professor of MP Shah Medical College Jamnagar. In this laboratory PG and Ph.D scholar of Kayachikitsa as well as of other departments of the Institution carried out the semen analysis of their research work themselves. These scholars when got the job at other Institutions they established Vajikarana/semen lab in their institutions and continued the research work on semen abnormality and male infertility and their management.

Since then a lot of data has been generated and presented in their respective theses. The review of these works showed that in discussion portion the scholars are not so accurate in interpreting their data as well as to draw conclusions.

It seems that it may be due to ignorance regarding the details of the applied aspects of the data. Hence a need is felt to detail the interpretation of the various values obtained by the different semen parameters.

The subject of Infertility has acquired global dimensions. Gone are the days when women alone bore the blame for being unable to conceive. With increasing educational standards and health awareness, the participation of both the couples has become mandatory in the infertility check-up. This has led to the revelation that males are equally responsible i.e., up to 50% in the reported cases of Infertility.

Sample Collection Method: Following oral instructions should be given to the patients regarding the collection of the semen sample:

Abstinence: High fluctuations are common in all semen parameters with varying duration of sex performances. The relation between sexual abstinence and sperm count is well established. An abstinence of 3 days is suggested to be the most appropriate with the minimal fluctuations, and hence should be followed strictly for sample collection.

Way of Collection: Masturbation is believed to be the best method of collection of semen as it facilitates the complete collection of the ejaculate and also minimizes the chances of contamination.

All patients should be instructed to clean their hands and glans penis thoroughly with running tap water without the use of soap before collection.

However, in case of failure to masturbate, ejaculation can be induced with a penile vibrator. Coitus interrupts can be allowed only in case the patient failed to ejaculate with the above methods. Collection should be done in a clean hygienic room adjacent to the laboratory irrespective of the method of collection.

Collection Time: Different timings of the day have their effect on the quality of semen (Valsamma, 1988). In order to avoid such variations, it is better to fix the time of collection of the semen sample between 8:30 A.M. and 10:00 A.M.

Containers: 50 ml glass beakers which is pre cleaned warmed and labeled are to be used for semen collection.

(1) Seminal Parameters

After collection, the semen samples should be examined at room temperature as follows:

Color: The color of the semen is noted after liquefaction against a dark background. Semen is whitish grey to yellowish and tends to be more yellowish with the longer abstinence.

Discoloration of the semen may point to genital tract infection. The semen appears white or yellow due to presence of leukocytes in it and reddish color indicates the bleeding. Blood present in semen is called as hemospermia which indicates acute bacterial prostatitis or even internal injury to genitourinary tract.

Odor: The odor of the semen is compared with flowers of chestnut or acrid. It may be due to the oxidation of spermine.

Coagulation and Liquefaction: Immediately after ejaculation the liquid semen coagulates. The coagulumtive enzyme in man originates in the seminal vesicles, therefore azoospermia with complete lack of a coagulation indicates agenesis of the seminal vesicle orocclusion of the ejaculatory ducts.

Coagulated semen liquefies automatically within 5 to 40 minutes of ejaculation, butnormal liquefaction time is considered as 20 - 30 minutes

As liquefying enzyme-seminine is produced by the prostate gland, therefore, if the seminal coagulum fails to liquefy, it indicates prostatic dysfunction and also its infection. The persistent coagulum may trap spermatozoa and restrict their motility.

Un-liquefied samples may be liquefied by repeatedly forcing it through a 20 G needle and then utilized for further examination.

The presence of mucous streaks, if any should also be recorded.

Volume: Liquefied sample should be transferred into a graduated centrifuge tube and measure the volume to the nearest 0.2 ml. Generally semen is measured between 1.5 to 4.5 ml.

Low volume of semen may be due to spillage during collection, less abstinence, partial retrograde ejaculation, ejaculatory duct obstruction, vas aplasia, chronic seminal vesiculitis.

Increased in semen volume may be due to prostatic infection or contamination with urine.

Viscosity: It is the rheological property of the semen. Gopala Krishan and Ananda Kumar (1989) described the needle and syringe method for measuring the viscosity of the semen. The normal viscosity ratio measured by this method should be less than 9.

The viscosity may also be quantified by adopting following scoring system i.e., by measuring the thread formed by the glass rod vertically lifted from the semen as follows:

No thread formation =0 score
Thread formation < 1 cm = 1 score
Thread formation < 3 cm 2 =score
Thread formation < 5 cm =3 score
Thread formation > 5 cm =4 score

Viscosity Up to grade 1 is considered as normal. Hyper viscosity indicates the presence of infection, anti-sperm -antibodies, accessory gland dysfunction and increased morphologically abnormal sperms. It also interferes with the sperm motility and concentration.

The hyper viscous semen sample can be made more uniform and easy to handle for further tests by passing through 21 gauge needle.

pH: The semen pH should be measured immediately after liquefaction on a calibrated pH meter. It can also be noted by spreading the liquefied semen sample on a pH paper strip with the glass rod and then comparing with the standard color. The normal pH values vary between 7.5 and 8.

A high pH (>8) is indicative of prostatitis, vesiculitis or epidiymitis.

The low pH (< 7.2) is indicative of possible chronic infection or presence of only prostatic fluid. Also correlate with absent fructose.

Microscopic Examination

Sperm Viability: Freshly ejaculated sperm cells move at a velocity of 75 µm/second while a high proportion of sperm cells from infertile men move at about 40 µm/second (Harvey, 1960 and barco 1984).

For the critical process of fertilization few sperms may be sufficient but they must be motile. Motility of the sperm should be examined under standard temperature conditions e.g. body temperature of 37^0 C. Traditionally, three parameters are assessed while evaluating the sperm motility viz. percentage of motile cells, type of motility and longevity.

Sperm viability > 60% unstained sperms.

A motility loss 10% to 20% within 3 hours is considered as within the normal range.

Quantitative motility is determined by counting 100 to 200 sperm cells, while avoiding the edges of the cover slip.

Sperm Motility: More than 50% spermatozoa showing SLP plus more than 25%showing RLP are considered as normal motility.

Semen is stained with eosin to differentiate viable (unstained) and non-viable (stained) spermatozoa. This is a static test to measure membrane integrity and increased dead sperms indicate infection.

Motility: One drop of well mixed semen is taken on a pre-cleaned, warmed glass slide and covered with a sterile 20 x 20 mm cover slip. The slide is then examined under 400x. The spermatozoa are scanned systematically and were included in one of the following 4 patterns of motility.

Rapid Linear Progressive (RLP) motility, Slow Linear Progressive (SLP) motility, Non-progressive (NP) motility and immotile (Imm) are recorded during the count.

A total of 200 spermatozoa are counted, graded and the percentage is recorded.

Sperm motility >50% progressive or > 25% RLP motility is a good motility.

Poor motility is indicative of testicular defect, epididymal dysfunction and infection.

Round cells (Particulate debris): Cells other than spermatozoa like white blood cells, epithelial cells, germ cells should be counted in 10 different fields of the wet mount and the mean is calculated and represented as percentage of particulate debris.

Less than 10% is considered as normal. Since it is difficult to distinguish pus cells from precursor cells, therefore this point should be taken in mind in cases of their excess reporting by the laboratory.

W.B.C. < 1 x 106 / ml is normal

Presence of abnormally high number of immature germ cells indicates testicular dysfunction and of high leukocytes indicates infection.

AmorphousMatter: Fine amorphous matter seen in the background of the wet slide apart from spermatozoa and non-spermatozoa cells are recorded as grades 0 to +4. The samples with grade 2 and above of amorphous matter indicates infection.

Agglutination: Agglutination means the viable motile spermatozoa sticking to each other either head to head, mid piece to mid piece, tail to tail or a combination of any of these, called mixed type. Agglutination of sperms with cellular debris is nonspecific. Agglutination is counted in 10 randomly selected fields and is represented as percentage.

<10% to <20%agglutination is normal

20% agglutinatedand above is indicative of infection or anti-sperm-antibodies.

Sperm Count: Sperm count may be done by using either hemocytometer or by using Makler Chamber (Makler, 1978) or any electronic counting methods.

Colorimetric and fluorometric methods for estimation of sperm concentration are also proposed but in most of the centers the hemocytometric method is used.

A rough estimate of the spermatozoa can be made from the wet mount seen under 400x and the dilution is made accordingly. Specific counting is carried out by using Haemocytometer.

More than 20 million/ml count of sperms is considered as normal, but normal count does not mean good quality. Very low count i.e. less than 10mil/ml or very high i.e. more than 250mil/ml may reflect poor quality.

Sperm Morphology: Morphology of human spermatozoa is classified either using bright field microscope with stained smear or under phase contrast microscope with wet preparations. Spermatozoa are characterized as normal and abnormal. Further classification is done as head abnormal, mid piece abnormal, tail abnormal and headless. Head abnormality of sperm includes double head, amorphous head, pin head, tapering head, round head etc.

Mid piece abnormality includes bent neck, thinned out middle piece etc and Tail abnormality includes double tail, stumpy tail, coiled tail etc.

A total of 200 spermatozoa are counted and each abnormality is represented as percentage.

More than 30% normal forms are considered as normal. A high number of morphologically abnormal forms are indicative of infection.

This parameter provides most significant information discriminating between fertile and infertile man. The presence of a single anomaly i.e. round head indicates genetic cause and for that alternate therapy method is to be adopted.

Sperm Functional Tests

None of the characteristic evaluated during routine analysis give the fertilizing ability of spermatozoa. Hence following sperm function tests are necessary which give the functional competence of spermatozoa.

Hypo-osmotic Swelling (HOS) Test: This test evaluates response of spermatozoa to hypo-osmotic stress. Basis of this assay is when viable sperms are exposed to hypo-osmotic media, there is an influ of liquid causing the tail to coil (Jayendran et al1984). H.O.S.: More than 60%spermatozoa with tail coiling is considered as normal. Decrease in HOS positive reaction indicates loss of viability, hence may be an indication of degenerative changes in sperm.

Slide Test for Acrosome Intactness: The acrosome of spermatozoa contains number of proteases which play a crucial role in the penetration of spermatozoa through outer investments of oocyte. Acrosome enzyme dissolves the protein. On the basis of which this method has been standardized (Gopalakrishnan et al 1994).

Acrosome intactness > 50% sperms having mean diameter of >530 micron

More than 50% spermatozoa with helos having mean diameter of 30 µm is considered as normal. This test is a good indicator of sperm's ability to penetrate theoocyte investments. Loss of acrosome, absence of acrosome or irregular acrosome may indicate the possibility of low or nil ability to fertilization.

In-vitro Nuclear Chromatin De-condensation (NCD) Test: One of the early events of fertilization of following the sperm penetration into the egg is the de-condensation of the sperm nuclear chromatin (Gopalakrishnan et al 1991). A good correlation has been shown between NCD and in vitro fertilization of human oocytes.

More than 70% de-condensed head is considered as normal. In cases with increased disturbances in chromatin material one should go back to the history for chemotherapy, radiation etc.

Sperm Mitochondrial Activity Index (SMAI): This test estimates the incidence of spermatozoa having a full complement of mitochondria containing respiratory enzyme which are essential for providing energy for the motility of spermatozoa. Lack of mitochondrial enzymes impair sperm motility and may thus contribute to sub-fertility (Gopalakrishnan et al 1990).More than 50 index is normal. This assay is useful to differentiate the asthenozoospermic sample into one with flagellar defects and those with mitochondrial defects. Disorder of motility, thereby alteration of SMAI may be associated with infection or pH alteration in the media used.

Biochemical/Fructose Tests

Fructose is the main source of glycolytic energy of sperm and its absence in semen indicates agnesisof seminal vehicles and vas or epididymal obstruction. More than 12 µmol/ml or 1200 µg/ml is considered as normal/ ??Fructose > 1200 microgm / ml. Seminal fructose level is used for the diagnosis of men with accessory gland dysfunction. Its concentration changes in number of pathological conditions such as cystic fibrosis obstruction of ampullo-vesicular tract.

Computer Assisted Semen Analysis (CASA)

Computer Assisted Semen Analyses (CASA) are available to perform automated semen analysis, including sperm counting, percentage of motile cells, motion analysis and determination of sperm morphology. A primary advantage of this equipment is that it provides a quantitative non-subjective assessment of the sperm motility. Infrared or video images are digitalized and analysed by processing algorithms th at determine the following properties of sperm motion:

1. Curvilinear velocity (VCL micrometer/second) is the overall distance travelled by the sperm divided by the time.
2. Straight line velocity (VSL micrometer/second) is the velocity measured in a straight line from the beginning to the end of the sperm track over time.
3. Linearity (LIN percent) is the ratio of VSL to the VCL.
4. Amplitude of lateral head displacement (ALH micrometer) is twice the maximum value of the distance between the actual position of the sperm head on the track and the average position.
5. Beat cross Frequency (BCF cycles/second) is the number of times the sperm head crosses the track path in either direction.

Sperm motion analysis are particularly useful when very large numbers of specimens (e.g. epidemiologic studies) are tested as thay can be video-taped for subsequent analysis. The data are then stored in database files and can be sorted and statistically analyzed with very little user's effort. An association between sperm motility pattern and fertility has been reported (Hunting et al 1988). CASA promises to be animportant tool in the evaluation of male infertility.

Testicular Biopsy: It is indicated in an infertile couple when the man on two separate occasions shows azoospermia or a sperm count is below 2mil/ml particularly if the motility of the spermatozoa is poor. This may also be done if the serum FSH is either normal or low and the infection in ihe prostatic or seminal vesicle is ruled out. The biopsy material is to be sent in Bouins solution (glacial acetic acid 5 ml + formalion 25 ml + saturated solution of picric acid 75 ml) and not in formalin or normal saline.

Sex Chromatin Study: In suspected cases where serum testosterone level is low and remains unresponsive even after daily injunction of HCG 5000 unit for four days.

Expressed Prostatic Secretion (EPS): In the patients whose semen revealed hyperviscosity, increased percentage of round cells, EPS examination was carried to rule out or diagnose prostatitis. After sexual abstinence of 2 days, prostatic massage was done in left lateral position per rectally, and the prostatic secretion expressed per urethra was collected on a precleaned glass slide. It was covered with a clean 20 x 20 mm cover slip and examined for presence of pus cells under 400x magnification. Presence of pus cells, amorphous matter is a sure sign of prostatitis.

SemenCulture: Semen samples were collected in specially provided sterile containers from microbiology laboratory, with all precautions to avoid contamination. The collected samples were then sent to the microbiology laboratory for culture test under IRR the supervision of a senior microbiologist along with the presence of scholar.

Culture was carried out using MacConky and nutrient agar, incubated at 37°C and examined initially after 24 hours for any growth and reassessed again after 48 hours.

Predictive Value of Semen Analysis

Semen analysis is only 60% accurate in prediction of infertility as many men with apparently poor quality of semen may father a child.

During first few years of marriage, occurrence of pregnancy is independent of semen quality (FertilSteril, 1989, 51:324).

The predictive value of semen analysis can be improved by correlation with the 'trying time' and results post coital Test (PTC).

The shorter the duration of infertility, the better are the chances of pregnancy, irrespective of semen analysis (British Journal of urology 1983, 15:774)

Men with low count and motility but a positive PCT have the same chance of impregnating their partners, as do men with normal count and motility.

Additional prognostic information may be acquired from sperm function test.

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