

# The Infertile Couple

## Part II. Examination and Evaluation of Semen

J. A. VAN ZYL

### SUMMARY

Most laboratories in South Africa have their own methods for examining and evaluating semen. This situation contributes towards conditions unfair to patients and undesirable for physicians dealing with infertility. The technique of obtaining semen specimens for examination is described. All tests that should be done as a routine and according to international standards, as carried out at Tygerberg Hospital, are described, with special reference to pitfalls in the examination and evaluation of semen. An urgent plea to maintain international methods, standards and terminology and for adequate specific training of technologists in the field of andrology is made.

*S. Afr. med. J., 57, 485 (1980).*

Although many clinicians are of the opinion that semen need only be examined when poor results are obtained with the Sims-Huhner test, semen examination is of paramount importance in the management of patients who complain of infertility. According to MacLeod,<sup>1</sup> of all the laboratory studies performed today, semen examination probably receives the greatest maltreatment, not only in performance but also in interpretation. This can be a tragedy for the patient and misleading for the physician. This being the opinion of a world authority, it is almost unbelievable that we still find practising physicians and laboratory personnel who examine and evaluate semen according to what can best be described as a self-made programme of investigations and evaluation of semen.

### THE SPERMIogram

A spermiogram is a laboratory report compiled from data obtained after a series of at least four semen examinations of each individual patient. It shows the mean value of each semen parameter and portrays the hormonal and reproductive capacity of the testes. No spermiogram can be considered complete and reliable as a base for prognosis unless the non-immunological and immunological tests, certain essential biochemical parameters of the seminal plasma, as well as *in vitro* and *in vivo* penetration and migration tests have been performed and evaluated according to the methods, standards and terminology accepted by the Comité Internacional de Andrologia<sup>2-4</sup> (Table I). Table I shows the minimum data that must be contained in an examination report of semen (spermiogram).

**Department of Andrology, Division Obstetrics and Gynaecology, University of Stellenbosch and Tygerberg Hospital, Parowvallei, CP**

J. A. VAN ZYL, M.B. CH.B., M.MED. (O. & G.), M.D.

Reprint requests to: Dr J. A. van Zyl, Dept of Andrology, Tygerberg Hospital, Tygerberg, 7505 RSA.

TABLE I. STANDARD INTERNATIONAL NORMAL VALUES FOR SEMEN PARAMETERS AND TESTS

Non-immunological parameters	
Colour	Opaque
Coagulation and liquefaction	$\leq 20$ min
pH	7.7 - 8.4
Volume	2.0 - 6.0 ml
Viscosity	0 - 30 mm
Motility	$\geq 40\%$
Agglutination	0 - $\pm$
Speed of forward progression (grade 0 - 4)	$\geq 3.0$
Motility index	$\geq 120$
Kremer test	
Control blood serum, cervical mucus	$> 5$ progressively moving spermatozoa at 50 mm after 2 h
Viability (supravital staining)	$\geq 50\%$
Sperm density/ml	$\geq 20$ million
Morphology	
Normal head, neck, middle piece and tail	$\geq 40\%*$
White blood cells and other cells	0 - $\pm$
Immunological tests	
MAR test	0 - $\leq 10\%$
SCMC test	After 30 min shaking phenomenon (S%) 0 - 30
Kibrick test	Negative
Franklin-Dukes test	$\leq 10\%$
Friberg test	Negative
Isojima and Koyama test	$\leq 2$
Hamerlynck and Rümke test	Negative
Biochemical tests	
Fructoset	$\geq 8.4$ mmol/l
Acid phosphatase†	$\geq 25$ mU/l
Zinc‡	$\geq 2$ $\mu$ mol/l

\* World Health Organization (1978) (I. D. Cooke — personal communication).

† New SI values.

‡ MAR = mixed antiglobulin reaction, SCMC = sperm cervical mucus contact.

### INSTRUCTIONS FOR OBTAINING SEMEN SPECIMENS

A 3-day period of continence is required from patients, mainly because this equals the average frequency of intercourse of couples in the age group seeking help.<sup>1</sup> It is erroneous to practise abstinence for more than 7 days in order to 'build up' semen, since abstinence increases the count per millilitre but has an adverse effect on semen

volume and sperm motility. As volume increases, quantitative and qualitative motility diminishes drastically.<sup>2</sup>

In our laboratories all semen specimens are obtained by massage of the penis, either by the patient or by his wife, and are collected in sterile, wide-neck, plastic vials with a screw top ( $4.5 \times 5.5$  cm) that have been tested for spermicidal substances. Glass containers cannot be used unless the temperature in the 'semen room' and the laboratory is kept between  $18^{\circ}\text{C}$  and  $37^{\circ}\text{C}$ , since this may result in asthenozoospermia and necrozoospermia caused by cold shock. The use of a rubber condom is not recommended, since it contains spermicidal substances which can cause asthenozoospermia and necrozoospermia. The only type of condom that can be recommended and which is necessary when patients find it impossible to ejaculate after penis massage or with the use of a vibrator is a plastic condom (Milex Corporation, Chicago), in which normal sperm motility is maintained for relatively long periods.<sup>1</sup> Plastic condoms are manufactured in a different way from rubber condoms and have a seam that may irritate the vagina and even the penis. Physicians should inform patients about this and instruct them to use petroleum jelly on the outside of the condom or to use an applicator or ordinary plastic syringe to put liquid paraffin into the vagina before coitus. Care must be taken that none of these substances comes into contact with the semen, since this will adversely influence semen parameters. Plastic condoms need only be used for the specific purpose of collecting semen for examination. Coitus interruptus usually results in spilling the first fraction of the ejaculate, which is most valuable since it contains spermatozoa of a better quality with regard to percentage motility, speed of forward progression and sperm morphology than the remainder of the ejaculate. Semen specimens produced at home and delivered to the laboratory afterwards are subject to contamination. The time lapse and exposure of semen to a temperature lower than body temperature also have a marked influence on motility and coagulation and make it difficult to observe liquefaction. For these reasons semen specimens not obtained in the semen room at the andrology laboratories are potentially influenced by adverse factors and cannot be considered ideal material for investigation. If the specimen is collected at home, it must reach the laboratory within 1 hour after ejaculation.

In order to make satisfactory arrangements with patients to obtain semen specimens, tactful discussions and an awareness of the embarrassment and tension to which patients are subjected are needed. Psychogenic influences such as those caused by psychosexual problems, stress, anxiety and embarrassment have a marked influence on semen parameters.<sup>3,4</sup> A common phenomenon is that the psychological inhibition experienced by many patients approached for the first time to produce a semen specimen or to produce a specimen at a time of emotional stress, as is often the case with infertile couples, causes the semen parameters to resemble those of azoospermia or oligo-asthenozoospermia, the so-called 'stress pattern'. Consecutive semen examinations of the same patient, carried out at different intervals, may then show a marked improvement and even a normal semen pattern.<sup>2-4</sup>

At no stage has the female staff anything to do with

patients who come to produce semen specimens. Appointments for use of the semen room and the handing out of vials and forms are taken care of by male technologists. Every precaution is taken to prevent tension and embarrassment. To counteract impotence, I conduct an open-hearted discussion with patients and motivate them by stressing the importance of semen examination. The incidence of reluctance or refusal to co-operate can be regarded as nil. Patients are instructed to wash their hands with soap and water and their penis without soap. In order to clear the urethra, patients urinate into a sterile container before ejaculating directly into a sterile vial. They are warned to take precaution against spilling the first fraction of the ejaculate, since this may result in retarded liquefaction, abnormal viscosity, a low motility, a low sperm count and a low content of prostatic phosphatase.<sup>3</sup> If the urine is cloudy, it is submitted for microscopic examination, culture and sensitivity tests.

A well-planned and well-equipped semen room next to the andrology laboratories eliminates most of the disadvantages usually connected with producing semen specimens on request. The semen room must be near the laboratories but isolated enough to secure privacy. It must be air-conditioned and furnished with a bed, an armchair, a toilet and a hand basin. As soon as a patient enters the semen room, he switches on a light which can be observed in the laboratory, and on finishing he switches off the light immediately before leaving. In this way no other person knows that there is a patient in the semen room and no one sees him leaving. Patients feel much more at ease in this way, and the laboratory staff know when to fetch the semen specimen.

## NUMBER OF SEMEN EXAMINATIONS

I never give a prognosis after completion of a single semen examination. In order to determine testicular function several semen specimens are obtained for examination at intervals, preferably over a period of 3 months at monthly intervals, since the cycle of spermatogenesis takes  $74 \pm 4$  days.<sup>5</sup> Spermatogenesis can be influenced by several factors and will result in a variation in semen parameters, which can lead to an erroneous diagnosis and evaluation, even if the first specimen is found to be probably fertile. In 20% of cases, follow-up semen examinations of specimens which are probably fertile reveal a variation in parameters. Chronic prostatovesiculitis is usually revealed after repeated semen examinations, and this alone justifies the routine of more than one semen examination per patient.<sup>4</sup> At least four semen examinations are done, after which the mean value for each parameter is calculated, even if the first semen specimen was evaluated as probably fertile. By regarding the mean values for four specimens, a more significant portrayal of the patient's semen quality can be obtained. In order to determine progress, follow-up semen examinations are carried out at later stages of treatment.

## NON-IMMUNOLOGICAL TESTS

Air-conditioned laboratories are essential. Before any *in vitro* tests are done, the temperature in the laboratories

must be controlled to ensure that it will not fall below 18°C or rise above 37°C.

## Colour

The colour of a semen specimen is observed macroscopically and written down at the beginning of the examination. Normal semen is opaque. If the colour varies to brown or red, this is suggestive of blood in the semen; if it is yellow, the possibility of prostatovesiculitis, urine in the semen, or the use of oral antibiotics must be kept in mind. The colour of semen is also influenced by the period of abstinence: the shorter the interval the more transparent it becomes, and after longer periods it becomes more yellowish. The distinctive odour of semen may be absent in prostatic atrophy and prostatovesiculitis.<sup>3</sup>

## Coagulation and Liquefaction

Although human semen is ejaculated as a moderately viscous substance, it coagulates immediately after ejaculation to form a gel. *In vitro* liquefaction of semen occurs spontaneously within 5 - 20 minutes. Small clots resembling boiled sago grains may be present during the first hour after ejaculation, but must be regarded as normal.<sup>3</sup> If any non-liquefied material is present after 1 hour, this suggests impaired prostatic function, usually as a result of infection. If there is no immediate coagulation or none at all, this is indicative of bilateral absence of the vas deferens and the seminal vesicles. Because of these findings semen specimens obtained elsewhere and brought to any laboratory even within 2 hours after ejaculation, are of no value for an ultimate observation of coagulation and liquefaction. As soon as coagulation and liquefaction have been determined, the semen specimen is poured into a 10 ml graded glass centrifuge tube to determine the hydrogen ion concentration and volume.

## Hydrogen Ion Concentration (pH)

Unless the pH determination is done immediately after liquefaction, the value obtained may be too low as a result of lactic acid formation. The routine procedure is carried out with a pH meter. Indicator paper should not be used, since the results are unreliable. The pH of normal, freshly ejaculated semen varies between 7,7 and 8,4. The longer the lapse of time after ejaculation, the lower the pH. This, together with the fact that in cases of chronic disease or obstruction in the male genital tract the pH is always below 7,0, points to the importance of obtaining semen specimens at the laboratories in order to determine whether a lapse of time, chronic disease or obstruction is an influencing factor.<sup>3,4</sup>

## Volume

The scanty portion of semen that will stick to the sides of the plastic container when the semen specimen is poured into the centrifuge tube will hardly influence the volume of the specimen, and if meticulous care is taken this slight waste can be disregarded. The volume as observed in the centrifuge tube is then noted.

Authorities in this field agree that a fall in volume below 1 ml may influence conception.<sup>1,3,4</sup> If the volume is less than 1,5 ml, it is not possible to carry out biochemical and immunological tests simultaneously with non-immunological tests; several subsequent semen specimens will then be required to carry out one complete semen examination.

For detailed semen examination, it is absolutely necessary to have a well-mixed semen specimen. To obtain this, a heterotator (Instruments AB, Solna, Sweden), which slowly and gently rotates the centrifuge tubes filled with semen for 10 minutes before the tests and keeps on rotating the specimens until all tests have been completed, is used. The gentle, rhythmic movement distributes all components evenly in a way that can never be equalled with any manual process; the heterotator thus aids in completely eliminating false high or low counts per millilitre. Another outstanding feature of the heterotator is that any tube can be removed without stopping the rotation of other tubes, thus eliminating uneven distribution until all investigations have been completed.

I have investigated possible adverse effects such as exposure to air and light, contact with the rubber sealing of the centrifuge tubes, and continuous movement which may occur during use of the heterotator. None of these factors has been proved to have the slightest adverse effect on any semen parameter. It is, however, essential that the centrifuge tubes should be sealed with rubber and not with cork, since the latter gives off particles that mix with the semen and cause artefacts. Ordinary cork also influences motility and facilitates agglutination.

## Viscosity

After 10 minutes on the heterotator, the viscosity of the semen is determined. Care must be taken not to confuse coagulation and viscosity. Viscosity is determined by means of a Pasteur pipette.<sup>2</sup> If semen is simply poured from the container into a graded centrifuge tube, small clots which cannot be observed macroscopically may be present and will influence viscosity. If there are any clots or non-liquefied material in the specimen 1 hour after ejaculation, the specimen must be centrifuged to obtain seminal plasma, of which the viscosity can then be determined. Different methods for this have been described in detail.<sup>2,4</sup>

Increased viscosity indicates abnormality, especially infection of the prostate; even an examination of the prostate and prostatic massage may increase viscosity.<sup>3</sup> Quantitative and qualitative motility is influenced by increased viscosity, since, in a substance of this consistency, spermatozoa show no forward progression but only move their tails. Thus, increased viscosity also impairs or prevents *in vivo* penetration and migration. A negative Sims-Huhner test may thus be due to increased viscosity.<sup>3</sup>

If the lack of viscosity becomes so obvious that semen resembles a watery solution which tends to spill freely from the pipette, viscosity can be considered as low. Low viscosity is observed in pathological conditions of the vesiculae seminales and bilateral absence of the Wolffian ducts. Semen of low viscosity, being more liquid than it should be, tends to flow freely in the vagina rather than to form a good seminal pool.<sup>3</sup>

As soon as the viscosity has been determined, portions of semen are removed from the centrifuge tube for further investigations.

### Motility

Three aspects of sperm motility must be considered. Quantitative motility (percentage) and qualitative motility (speed of forward progression) must be estimated according to the method of MacLeod,<sup>1,6,7</sup> *in vitro* sperm penetration must be carried out according to Kremer's<sup>8</sup> method, and the viability of spermatozoa must be examined as described by Eliasson.<sup>9</sup>

Spermograms received from all over this country and observed by me at several laboratories in other countries suggest that sperm motility is unfortunately not regarded as significant enough to compel clinicians to examine it thoroughly. Motility is of paramount importance, since patients with an adequate sperm count per millilitre but impaired motility and those with a relatively poor count per millilitre but exceptionally good sperm motility deserve careful evaluation to determine a prognosis. This is why it is altogether inadequate to report that any semen specimen is 'non-motile' (poor), 'moderately motile' (moderate/adequate) or 'very active motile' (good/excellent). Evaluations such as these have no clinical or statistical value and give rise to confusion.

The evaluation of quantitative and qualitative motility can only be carried out by clinicians and technologists who have been trained by MacLeod or who have worked in laboratories where staff trained to use MacLeod's method are available to teach his standards for evaluation. By means of repeated semen examinations I have found that patients referred to me with spermograms which report a motility of 80% and a forward progression from 3+ to 4 were evaluated erroneously; motility of this exceptional quality is rare, even in a general population.

Because seminal plasma serves only as a temporary medium for spermatozoa in transition, semen smears incubated in Petri dishes are no longer used to determine motility at intervals over a period of 24 hours. These smears have the advantage, however, that specific agglutination can be observed at intervals (usually after 2 hours) and that infection which could have been concealed during earlier investigations can be traced.

The Kremer<sup>8</sup> test shows the depth of sperm penetration, penetration density, and the duration of sperm motility in control blood serum as well as in the wife's cervical mucus, provided the quality of the semen and of the cervical mucus is within normal limits. Therefore this test can be applied as a screening test for both semen and cervical mucus.<sup>8</sup>

I have found that *in vitro* sperm penetration tests correlate well with what can be expected to happen *in vivo*, unless the cervical mucus reacts in a hostile manner. Furthermore, if the spermatozoa in the control blood serum can penetrate 60 mm in 2 hours at 37°C, these spermatozoa can be expected to have an arbitrary *in vitro* speed of 3. To differentiate between immobile and dead spermatozoa, the supravital staining method of Eliasson<sup>9</sup> must be applied. With this method living spermatozoa

(bluish) and dead spermatozoa (yellow) can be calculated and expressed as a percentage (counting 200 cells). Therefore, it is also the only means of diagnosing necrozoospermia.

### Sperm Density

The current international method to determine sperm density is to use a white cell pipette and a haemocytometer. The technologist handling the white cell pipette for spermatozoa counts must be very skilful, since drawing up semen and dilution medium inaccurately in this delicate apparatus may yield false counts. I apply my own modified method for counting spermatozoa.<sup>2,4</sup>

By using the standard glass tuberculin syringe (micromatic) as I described in detail and later revised to include the correction factor ( $X = 0,025 \text{ ml}$ ), sperm density can be reliably and accurately determined. Correlation of the results obtained with the tuberculin syringe and the Makler<sup>10</sup> chamber for rapid sperm counts reveals significant advantages.

Although the sperm count per millilitre is not of paramount prognostic importance, it is the parameter according to which patients' semen is classified, and remains the parameter which can be explained easily to patients to offer them a relative understanding of their prognosis.

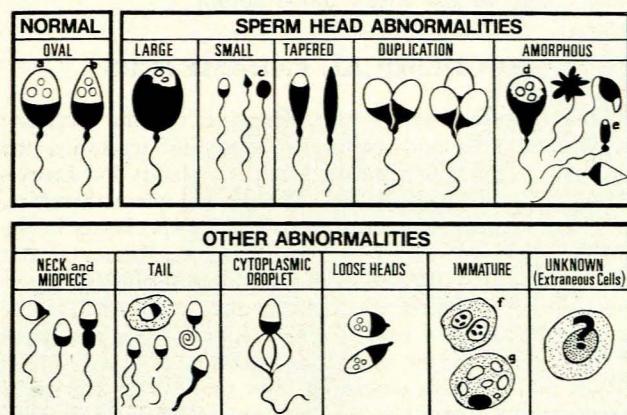
### Morphology

The morphological classification of sperm can be determined accurately by using an optimal staining method and selective classification, but this parameter remains the most difficult to determine. According to Eliasson,<sup>11</sup> criteria for 'normal' spermatozoa have not been so defined that scientists can adopt the same principle for deviation between 'normal' and 'pathological' cells. The lack of uniformity with regard to classification and the different staining techniques add to the problem.

Only well-trained and experienced technologists by international standards can differentiate between 'normal' and 'pathological' cells. Since the thickness of the smear determines whether spermatozoa will form layers and will in this way make it impossible to observe each cell individually, completely and precisely, a specific thickness is essential. An optimal smear will show each spermatozoon in such a way that its acrosome, nucleus, neck, middle piece and tail show up clearly and well-stained so that they can be measured, evaluated and classified. In order to eliminate erroneous calculations, the staining of the smear must also clearly show the cytoplasm of all other cells, to differentiate between immature cells, white blood cells and extraneous cells (Fig. 1). No other staining method equals the Papanicolaou technique in these respects.<sup>12</sup>

In all stages of the Papanicolaou staining technique, careful judgement and skill are required, but three stages can be singled out as demanding exceptional care in order to secure an optimal colouring effect of the spermatozoa and immature cells: the haematoxylin stage, the EA 50 stage, and the mounting stage. During the haematoxylin stage the nuclei of all cells are stained. The haematoxylin batch must be filtered daily and replaced frequently, especially if it discolours. Unless this is done, the result

will be unsatisfactory staining of the nuclei which, in turn, will lead to erroneous identification of cells.



**Fig. 1.** Schematic representation of the internationally accepted morphological classification for sperm. Oval, large, small, tapered, duplication, amorphous and immature groups are classified according to MacLeod's<sup>1</sup> classification; neck and middle piece, tail, cytoplasmic droplet (must not be larger than half of sperm head) and loose head groups classified according to Eliasson's<sup>11</sup> classification; unknown (extraneous) cells classified according to my classification: normal oval spermatozoon in antero-posterior position (*a*), and in lateral position (*b*); small group includes round-headed, pyknotic spermatozoa without acrosome (*c*); amorphous group includes pear shaped (*d*), dumb-bell shaped (*e*) and other bizarre spermatozoa; and immature group includes spermatocytes (*f*) and spermatids (*g*). It is extremely difficult to differentiate between normal lateral, tapered, borderline amorphous, immature and unknown cells, unless a micrometer is used.

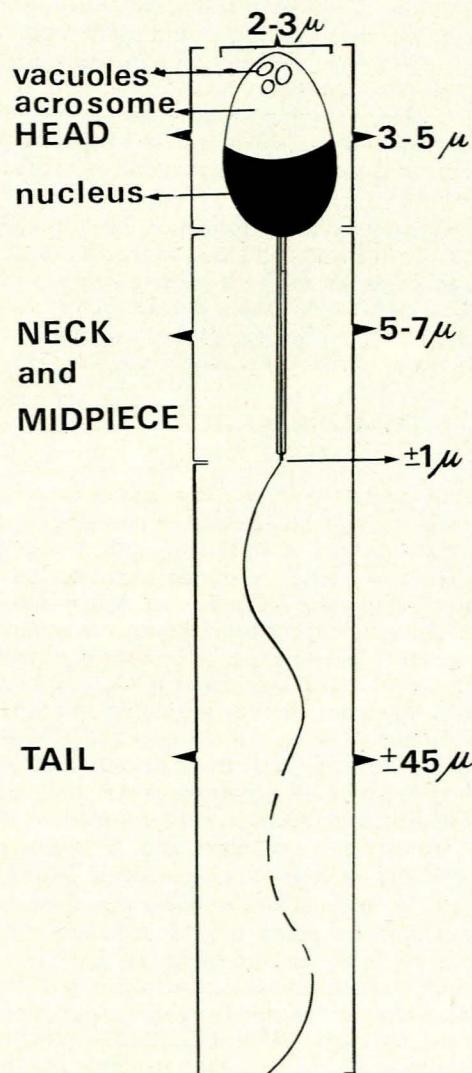
Cytoplasm should take on a pink (eosinophilic cells) or a green (cyanophilic cells) colour during the EA 50 stage. Unless vivid colouring in either of these studies is manifested, it will be impossible to observe and identify the neck, middle piece and tail of the spermatozoon. During the staining period smears must be submerged completely for the specified time, and after use the lids of all containers must be replaced firmly.

Depending on the staining effect obtained for the nucleus, neck, middle piece and tail, the duration of submergence can be adapted until a satisfactory result has been achieved, a technique that should be applied when staining semen slides, as spermatozoa do not lend themselves readily to staining effects. During the mounting stage, care must be taken not to expose the slides to water. The Canada balsam must only be on the edges of the cover glass, which must then be pressed down firmly to prevent discolouration of the slide during storage.

My morphological classification of sperm is based on a classification by MacLeod<sup>1</sup> and a more detailed classification of the neck, middle piece and tail by Eliasson,<sup>11</sup> both of which have been accepted internationally (Fig. 1). I have found it necessary to add an additional group to these classifications in order to differentiate between immature and extraneous cells (Fig. 1). Of the latter, numerous ones

can be found in the male genital tract in pathological conditions. It is of diagnostic value to have an exact calculation of immature and extraneous cells, since more than 3% of immature cells can be an indication for ligation of the internal spermatic vein, irrespective of the clinical diagnosis of a varicocele.

Semen smears must be examined under a 100 times magnification in oil with a Zeiss microscope equipped with a built-in micrometer so as to distinguish between normal sperm heads and deviations of the head. If there is doubt, the sperm head must be measured accurately before classification.<sup>11</sup> Special attention must be given to the size and characteristics of the acrosome. The size of each part of the spermatozoon must be measured with a micrometer and deviations of the acrosome must be noted (Fig. 2).



**Fig. 2.** Measurements of a normal spermatozoon.<sup>11</sup> Border-line deviations can be determined under a 100 times magnification in oil with a Zeiss microscope equipped with a built-in micrometer. The size of a normal acrosome is approximately half of the sperm head.<sup>18</sup>

The exact number of cells that must be counted and the method of counting have been described in detail.<sup>2</sup>

The morphological classification of sperm is the only parameter of which the results can be compared by interchange among laboratories working according to international standards, thus enabling scientists to keep up their standard of semen examination. A minimum period of 3 months is required to learn the rudimentary steps for the determination of sperm morphology and 1 year of training to acquire the skill to evaluate this parameter accurately. This is also applicable to sperm motility.

The current method to determine azoospermia is by a wet smear. A drop of semen is placed on a slide and covered with a cover glass (the drop method) before microscopic scanning. Since only one drop of potentially azoospermic semen is used, the diagnosis will be more accurate if the complete ejaculate is centrifuged with a Shandon-Elliott cytocentrifuge until only approximately one drop is left. This method of centrifuging is extremely effective if a Shandon-Elliott centrifuge is used.<sup>2</sup> The smear must be stained according to the Papanicolaou method to ensure a good staining effect and so to detect spermatozoa.<sup>12</sup> Supravital staining must be done to eliminate dead spermatozoa.<sup>9</sup>

The current accepted incidence of azoospermia is unrealistically high, probably due to the fact that semen is centrifuged by other methods.<sup>2</sup> The Shandon-Elliott cytocentrifuge shows that erroneous diagnosis of azoospermia is often due to the fact that spermatozoa cannot be observed on a wet smear. True azoospermia is rare.

## IMMUNOLOGICAL TESTS

Since 1976 I have routinely done the direct mixed antiglobulin (MAR) test<sup>13</sup> and the sperm cervical mucus contact (SCMC) test<sup>14</sup> (J. Kremer — personal communication) for the detection of antisperm antibodies in men and women. These two tests are simple screening tests for the detection of antisperm antibodies in semen and cervical mucus. The highly sophisticated antisperm antibody tests, i.e. the gelatin agglutination test (Kibrick, modified by Shulman), the tube-slide agglutination test (Franklin-Dukes, modified by Shulman), the tray agglutination test (Friberg), the sperm immobilization test (Isojima and Koyama), and the spermocytotoxicity test (Hamerlynck and Rümke),<sup>15</sup> are applied in cases of a positive MAR test, a positive SCMC test, asthenozoospermia, necrozoospermia, oligozoospermia, azoospermia, a poor and a negative Sims-Huhner test and in cases of long-standing infertility.

Especially in cases of long-standing (unexplained) infertility care must be taken not to pronounce semen as possibly fertile unless the screening test and the complete series of highly sophisticated antisperm antibody tests have been done. Even with negative screening tests, these highly sophisticated tests can show up antibodies. On the other hand, all specimens of probably infertile and probably sterile semen will not have antibodies. The same applies to cervical mucus. Telang *et al.*<sup>16</sup> have found that with an excellent Sims-Huhner test, 7% of women and 21% of men showed the presence of antibodies. On the other hand, in groups with a poor Sims-Huhner test, 48% of women had

no antibodies. These findings indicate the importance of simultaneous screening tests on the husband's semen and highly sophisticated antisperm antibody tests on the husband's and on his wife's blood serum.

## BIOCHEMICAL PARAMETERS

Biochemical parameters are indicative of the secretory activity of the glands in the male genital tract, i.e. the seminal vesicles, the prostate, Cowper's glands and Littré's glands. The biochemistry of semen is complex; therefore, a variety of tests have been described by specialists in the biochemical aspects of semen. However, Eliasson<sup>9</sup> has selected and described meticulously the tests for the determination of fructose, acid phosphatase and zinc as the most important for routine investigations. The results of these tests reflect the endocrine function of the germinal glands and serve as screening tests for lesions, especially infection, of the male genital tract. Raffi *et al.*<sup>17</sup> have described the acrosin activity of morphologically normal and abnormal spermatozoa. During the evaluation of

SPERMIOGRAM	
DEPARTMENT ANDROLOGY, TYGERBERG HOSPITAL	
SURNAME AND INITIALS :	DATE OF BIRTH :
HOSPITAL NUMBER :	REFERRED BY :
Date :	Specimen obtained by :
Volume (ml) :	Period of abstinence : days
Coagulation (minutes) :	Colour :
Liquefaction (minutes) :	Viscosity :
Count/ml (million) :	Leucocytes :
Total count (million) :	Erythrocytes :
Motility ( $37^{\circ}\text{C}$ ) :	Miscellaneous cells :
Supravital staining :	Agglutination :
Forward progression ( $37^{\circ}\text{C}$ ) :	Spermine phosphate crystals :
Morphology (normal spermatozoa) :	Urine : culture and sensitivity :
Total : normal spermatozoa/ejaculate :	BIOCHEMICAL TESTS
Culture and sensitivity :	Fructose mmol/l :
KREMER TEST (SPM) :	Acid phosphatase MU/l :
Control serum (mm) :	Zinc $\mu\text{mol/l}$ :
Wife's serum (mm) :	SIMS-HUHNER TEST (2 hours post-coital)
Wife's cervical mucus (mm) :	spermatozoa per high power field
Control cervical mucus (mm) :	total motility
Husband's serum (mm) :	forward progression
RATING :	
Normozoospermia	
Oligozoospermia	
Asthenozoospermia	
Necrozoospermia	
Teratozoospermia	
Polizoospermia	
Azoospermia	
Hypospermia	
Hyperspermia	
Aspermia	
IMMUNOLOGICAL : CLASSIFICATION (in vitro)	
probably fertile ejaculate	probably infertile ejaculate
probably sterile ejaculate	
REMARKS :	

TECHNOLOGIST : HEAD OF DEPARTMENT :

Fig. 3. A spermogram: an example of a report that I compiled of all *in vitro* and *in vivo* semen tests. The data represent the mean value of each parameter, calculated from findings after several semen specimens have been examined at intervals. In the space for remarks, all abnormal findings are noted.

sperm morphology the acrosome must be scrutinized because its characteristics may indicate the need to determine the acrosin level, which decreases markedly when there is no acrosome. If acrosin activity is impaired, spermatozoa may be prevented from penetrating the zona pellucida of the ovum. Even morphologically normal spermatozoa may have an acrosin deficiency.<sup>17</sup> In these cases an excellent Sims-Huhner result may be obtained, but fertilization will not occur.

On completion of all the non-immunological, immunological and biochemical tests, and the Sims-Huhner test, the average values of the serial tests are compiled and presented as a spermogram which forms the base for evaluation and classification of semen and for the prognosis and treatment (Fig. 3). Follow-up spermograms, which must be compiled at intervals, show the effect of treatment. Spermograms compiled within the first 3 months after conception can be regarded as fertile, irrespective of the values of the different semen parameters and of the previous classification of the semen. Follow-up spermograms are of academic and clinical value and should be compiled if possible. Semen should not be classified as 'fertile', 'subfertile', 'infertile' or 'sterile' until conception has occurred or if it has not been achieved. Until conception has occurred, preference must be given to 'probably fertile', 'probably infertile' or 'probably sterile'.

## CONCLUSION

The ignorant and negligent examination and evaluation of any semen parameter can have far-reaching consequences. These tests should be assigned to physicians and technologists specially trained in andrology. Trained personnel ensure standard results which can compare with those of any international laboratory, and reports from different

laboratories can be interchanged. Any laboratory that accepts semen specimens for examination must have the equipment to enable technologists to comply with the specific requirements for semen examination. There is reason to believe that only training hospitals can afford the expensive equipment needed for semen examination up to international standards. No clinician is entitled to pronounce on a patient unless the findings have been based on laboratory procedures which equal international standards and have been evaluated correctly.

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