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andro-test© and Semen Self-Exam: their importance in prevention and in the diagnostic process

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Abstract
The andro-test is the only semen analysis which describes the health condition of the male genital tract and the only one qualified for diagnostic purposes. The physiological values defined are used to evaluate the relative risk mainly of having a urogenital tract infection. This study assesses the importance of the andro-test and of the Semen Self-Exam in the preventive checkup of men and in the andrological diagnostic process. It is proven that in almost 9 out of 10 cases (87,68%), if at least one of the semen parameters is outside the physiological values, a microbe is detected in the semen sample in exam. Inversely, it is also proven that in 98,76% of the samples where a microbe was detected, at least one of the parameters of the andro-test was outside the physiological values.

Keywords. andro-test; sensitivity; specificity; positive predictive value; negative predictive value; SpermLab's criteria; semen analysis; semen self-exam; semen physiological values; annual andrological checkup; urogenital infections; semen pathology; sperm morphology; men's health.

Introduction

The andro-test is the semen analysis assessing the health condition of the male genital tract. The physiological values of each semen parameter have already been defined and the odds ratios between samples presenting abnormal values and those presenting physiological values have been estimated (Voulgaridis, 2018). The present study is supplementary to the study that led to the development of the andro-test. It uses the data from the same semen analyses included in the previous study, but it focuses in the assessment of the diagnostic value of the andro-test through the evaluation of the sensitivity and the specificity of each of the semen parameters and of specific sets of them, in order to decide whether the andro-test is the semen analysis appropriate for the assessment of men's health.

1. Revision of the semen analysis

In the last two editions of the W.H.O. manual on the analysis of semen (World Health Organization, 1999)(World Health Organization, 2010), it is denoted that the reference values shall not be used for diagnostic purposes. The W.H.O. admits that although these values were defined using data from different laboratories, these laboratories were not using the same methodologies in the assessment of semen parameters; neither were they assessing the same parameters. Indeed, the reference values of some parameters were evaluated by using the data from 400 analyses and some others by using the data from 1900 analyses. From a statistician's point of view, the previously mentioned procedure used for the definition of these values renders the W.H.O.'s reference values undoubtedly unreliable. The explanation that the W.H.O. provides in the 4th edition is characteristic: '*It should be noted that it is not the purpose of the manual to establish the minimum or lowest semen values compatible with achieving a pregnancy, in vivo or in vitro. … Reference ranges for human semen present some conceptual difficulties. The relationship of semen quality to fertility is complicated by many other factors, including female fertility. Thus men with abnormal semen may still be fertile while men with better than average semen quality produce pregnancies at higher than average rates. … . Finally, it should be emphasized that the major purpose of this manual is to encourage the use of standard procedures to establish reference values (previously called 'normal' values) for semen analysis*'.

Despite the fact that the W.H.O. has clearly denoted the limitations of these reference values, the clinicians use these reference values to make a diagnosis. The gynaecologists use these values to decide whether a man's semen is fertile or not. Surprisingly, what is even more worrying is the

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fact that these values are also used from the urologists in order to decide whether to treat a varicocele or not.

As already denoted by the W.H.O., these values depend on extrinsic factors, such us '*her fertility*'. Obviously, reference values that depend on extrinsic factors are not appropriate for the assessment of a condition that characterizes the male factor itself.

A semen analysis shall refer to intrinsic factors. In order to define the '*physiological values*' of each semen parameter, the assessment shall refer to the physiological function of the male genital tract.

The W.H.O. is aware that non-specialized laboratories lack proper training in semen analysis, while the IVF laboratories have a lack of interest. Indeed, the embryologists are only interested in whether there are enough spermatozoa, in order to go for an IUI, or only few, in order to go for an IVF (or ICSI). The gynaecologists are only interested in deciding between an IUI or an IVF, no matter if a high pH indicates that there is an inflammation of the male accessory glands. Ironically, the urogenital tract infections do affect the IVF's (or the pregnancy's) outcome. In addition, in the introduction of the 4th edition, the W.H.O. denotes the increasing incidence of urogenital abnormalities and testicular tumour. This is an observation that should arouse the public concern.

The problem stems from the fact that the semen analysis has always been considered as being a fertility test.

A semen analysis is not just about a 'sperm count'. Considering that the semen liquid is produced by the accessory glands, it becomes obvious that the physicochemical characteristics are not less important in the assessment of the health condition of the male genital tract. A semen analysis is therefore an essential tool for the urologist in the assessment of men's health. Therefore, we have to consider revising the assessment of human semen under the prism of the male genital health. We need to look into the diagnostic value of the andro-test.

Comparing the andro-test to 'the fertility test' described in the W.H.O.'s manuals, this recently introduced semen analysis was developed by studying the data from 1197 analyses meeting strictly standardized criteria used to minimise the factors that could bias the reliability of the conclusions extracted. The observational study was designed by biostatisticians and the assessment of each semen sample was performed according to the recommendations of the W.H.O.'s manual (4th and 5th edition) only by biologists specialised in Spermatology, in a Spermatology laboratory. The study was based on samples that had already been analysed, in order to avoid bias due to the researcher's partiality. Only the analysis from the first visit of each examinee was used, in order to avoid the bias due to incomplete treatment in the next analysis of the same examinee. Finally, the study included only samples on which a complete microbiological exam was performed and this microbiological exam was performed by an independent laboratory. It was the health condition of the male genital tract to be investigated, therefore, using intrinsic criteria for the evaluation of the physiological values of each parameter of human semen. Furthermore, the physiological values of the andro-test are related to specific pathological conditions of the male genital tract and the odds ratios for each parameter are accurately defined (see table 8.3.2 pag.38, Voulgaridis et al., 2018, https://doi.org/10.30551/ijs.v1i1.1). Limitations are described in the concluding remarks of the study and the author encourages the performance of future studies on individuals with a known andrological history, which is expected to refine the results and improve our knowledge in men's health.

The andro-test permitted the development of the '*Semen Self-Exam*', a method that permits every man, without the need of special training or laboratory equipment, nor any cost, to check his genital tract health regularly, at the comfort and discretion of his personal space. This self-exam is expected to raise the awareness of men, all around the world, in the importance of the preventive andrological checkup. In order to guarantee that this preventive checkup will be performed in a standardized way, it is given the name of '*Annual Andrological Checkup*'. In regard to the improvement of services to promote the global health, the authors denote the importance of the andro-test in the clinical practice and the prevention of serious pathological conditions in men, women and children.

Concluding, the investigation of standardising the procedures of the assessment of the fertilizing ability of the human semen shall be considered of secondary importance. The investigation of the importance of assessing the semen parameters under the prism of the overall health of the male genital health shall be adopted, instead. The *SpermLab's Criteria* and the new classification of morphological abnormalities described in the study of the andro-test, shall become a fundamental guideline to future studies.

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2. Diagnostic vs screening tests

A diagnosis is the judgment about what a particular illness or problem is. In the clinical practice, this is usually a complex process, which depends mainly on the competency of the clinician, the accuracy of the laboratory and the imaging exams. The clinician makes the diagnosis with respect to the medical history, the clinical examination, some imaging and laboratory exams. On the contrary, a diagnostic test usually uses a single parameter to predict a condition and shall be able to accurately distinguish between the positive and the negative result. Ideally, there should not be any false positive nor any false negative result. In practice, the less the false results are, the more accurate the diagnostic test will be. Often enough, a certain parameter may be related to more than one condition, independent of one another. Similarly, a certain condition may influence more than one parameter, independent of one another. In such cases, the diagnostic process includes an initial less specific test on a parameter, followed by other tests able to discriminate between the possible conditions.

A screening test is highly sensitive, but may be less specific. This means that whenever the test is positive, at least one of the related conditions actually exists. It also means that whenever one of the related conditions exists, the screening test is actually positive. However, in the screening tests' case, the vice versa is not true. This means that whenever a specific related condition does not exist, the screening test is not necessarily negative.

The semen analysis andro-test is a screening test. It is only a part of the diagnostic process through which the competent clinician, the urologist, is investigating the health condition of the male genital tract.

3. Assessment and evaluation of the diagnostic value of the andro-test and each of its parameters

3.1 Materials and methods

The same database used in the development of the andro-test was used here for the evaluation of the sensitivity and specificity of this test. Given that the andro-test consists of the assessment of several semen parameters, it was estimated the prognostic value, initially, of each one of these parameters separately and then, of specific sets of them.

The limitations of the database shall be reminded. Inevitably, limitations exist in every observational study. The reason for having a semen analysis was usually the subfertility, less often a check after treatment and rarely a check for an infection of the urogenital track or for a varicocele. This is due to the fact that, before the development of the andro-test, the interpretation of semen characteristics was made in relation to the likelihood of achieving a conception. This explains why the vast majority (80%) of the examinees was between 30 and 45 years old. Since it is very probable that a man until his thirties has already had a urinary tract infection (UTI), has not realised that he has this infection and he has not been properly treated so far, it is expected to find most of the samples infected. This explains why 87% of the samples where indeed found infected by at least one microbe. This fact overshadows other pathological conditions. For example, in order to evaluate the influence of varicocele on semen parameters, one has to study samples free of infections. Being an observational study, samples presenting indications of an infection were checked for microbes, but those not presenting them were not checked because the patient would have been charged unreasonably.

The data were analysed using Stata™ (Version 10.1 MP, Stata Corporation, College Station, TX 77845, USA). The semen samples were sorted according to the physiological values defined for the andro-test. This sorting was represented in two by two tables between the parameter used as a test parameter, and the event used as a criterion for the assessment of the diagnostic value of the test parameter. From these tables, it was calculated:

- the *sensitivity*, calculated as True Positives (TP)/ (TP + False Negatives (FN));
- the *specificity*, calculated as True Negatives (TN)/ (TN+ False Positives (FP));
- the *positive predictive value* (PPV), calculated as TP/ (TP+FP);
- the *negative predictive value* (NPV), calculated as TN/ (TN+FN);
- the *percentage of correctly classified* samples or *accuracy* (ACC), calculated as (TP+TN)/(TP+TN+FP+FN);

The area under the Receiver Operating Characteristic curve (also called AUROC) is reported, despite the fact that its reliability as a comparative measure of accuracy between the models' results has been put into question (Lobo, et al., 2007). This measure equals the mean between the sensitivity and the specificity.

From 1197 semen samples included in this study, in 152 (12,7%) no microbe was detected and for this reason these samples were categorized as controls. In 1045 (87,3%) cases (patients) at least one bacterium was detected.

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3.2 Physicochemical characteristics

The seminal liquid is produced mainly from the seminal vesicles and the prostate. It is thus obvious that variations in the function of these glands will be reflected in the composition and consequently in the physicochemical characteristics of the seminal liquid.

***Semen volume*.** An abnormal semen volume may be the result of an active exudation of the glands or an obstruction of the ejaculatory gland. However, a normal semen volume may also result from a decreased secretion of a gland compensated by an increased secretion of the other. Therefore, although an abnormal semen volume may be indicative of a pathological condition, a semen volume inside the physiological values does not guarantee the absence of microbes.

The relation between the semen volume and the presence of a microbe in the semen sample is proven to be statistically important. Indeed, it is proven to be 1,50 times more likely to detect a microbe when the semen volume is smaller than 2,4ml or bigger than 5,0ml (p-value=0,029).

At first, it was checked whether the semen volume in itself could predict the detection of a microbe in the sample (Table 3.1).

Table 3.1. Sorting between the semen volume and the presence of a microbe

Volume	Microb. exam		Total
	-	+	
-	105	625	730
+	47	420	467
Total	152	1045	1197

It was observed that in 89,94% of the samples where the semen volume was outside the physiological values, a microbe was detected (PPV). However, in 14,38% of the samples where the semen volume was inside the physiological values, no microbe was detected (NPV).

It was also checked if the detection of a microbe in the sample could predict whether the semen volume is inside or outside the physiological values.

It was observed that in 40,19% of the samples where a microbe was detected, the semen volume was outside the physiological values (sensitivity test). However, in 69,08% of the samples where no microbe was detected, the semen volume was inside the physiological values (specificity test).

The accuracy of a test using the semen volume to distinguish between the infected samples and those without a microbe is 43,86%, which corresponds to the percentage of the correctly classified samples in table 3.1. The area under the ROC curve equals 0,5216.

***Semen pH*.** The relation between the semen pH and the presence of a microbe in the semen sample is proven to be statistically important. Indeed, it is proven to be 2,00 times more likely to detect a microbe when the semen pH is lower than 7,7 or higher than 8,3 and this difference is proven to be statistically significant (p-value<0,001).

At first, it was checked whether the semen pH in itself could predict the detection of a microbe in the sample (Table 3.2).

Table 3.2. Sorting between the semen pH and the presence of a microbe

pH	Microb. exam		Total
	-	+	
-	106	559	665
+	46	486	532
Total	152	1045	1197

It was observed that in 91,35% of the samples where the semen pH was outside the physiological values, a microbe was detected (PPV). However, in 15,94% of the samples where the semen pH was inside the physiological values, no microbe was detected (NPV).

It was also checked if the detection of a microbe in the sample could predict whether the semen pH is inside or outside the physiological values.

It was observed that in 46,51% of the samples where a microbe was detected, the semen pH was outside the physiological values (sensitivity test). However, in 69,74% of the samples where no microbe was detected, the semen pH was inside the physiological values (specificity test).

The accuracy of a test using the semen pH to distinguish between the infected samples and those without a microbe is 49,46%, which corresponds to the percentage of the correctly classified samples in table 3.2. The area under the ROC curve equals 0,5365.

***Semen liquefaction*.** The relation between the semen liquefaction and the presence of a microbe in the semen sample is proven to be statistically important. Indeed, it is proven to be 1,46 times more probable to detect a microbe in a sample not liquefied in half an hour from collection compared to those liquefied in these first 30 minutes and this result is proven to be statistically significant (p-value=0,034).

At first, it was checked whether the semen liquefaction in itself could predict the detection of a microbe in the sample (Table 3.3).

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Table 3.3. Sorting between the semen liquefaction and the presence of a microbe

Liquefaction	Microb. exam		Total
	-	+	
-	93	543	636
+	59	502	561
Total	152	1045	1197

It was observed that in 89,48% of the samples where the semen liquefaction was delayed, a microbe was detected (PPV). However, in 14,62% of the samples where the semen liquefaction was normal, no microbe was detected (NPV).

It was also checked if the detection of a microbe in the sample could predict whether the semen liquefaction is normal or delayed.

It was observed that in 48,04% of the samples where a microbe was detected, the semen liquefaction was delayed. However, in 61,18% of the samples where no microbe was detected, the semen liquefaction was normal.

The accuracy of a test using the semen liquefaction to distinguish between the infected samples and those without a microbe is 49,71%, which corresponds to the percentage of the correctly classified samples in table 3.3. The area under the ROC curve equals 0,5205.

Semen viscosity. The relation between the semen viscosity and the presence of a microbe in the semen sample is not proven to be statistically important, exception made for the condition in which the semen volume and the semen pH are both inside the physiological values. In this special case, it is proven to be 1,95 times more probable to detect a microbe in a less viscous sample compared to a sample presenting normal viscosity and this result is proven to be statistically significant (p-value=0,05). Therefore, only this special case is investigated.

At first, it was checked whether the semen viscosity could predict the detection of a microbe in a sample where the semen volume and the semen pH are inside the physiological values (Table 3.4).

Table 3.4. Sorting between the semen viscosity and the presence of a microbe

Viscosity	Microb. exam		Total
	-	+	
-	34	143	177
+	14	115	129
Total	48	258	306

It was observed that in 89,15% of these samples where the semen was watery, a microbe was detected (PPV). However, in 19,21% of these samples where the semen viscosity was normal, no microbe was detected (NPV).

It was also checked if the detection of a microbe in the samples where the semen volume and the semen pH are inside the physiological values could predict whether the semen viscosity is normal or lower than normal.

It was observed that in 44,57% of these samples, the semen was watery. However, in 70,83% of these samples where no microbe was detected, the semen viscosity was normal.

The accuracy of a test using the semen viscosity to distinguish between the infected samples and those without a microbe is 48,69%, which corresponds to the percentage of the correctly classified samples in table 3.4. The area under the ROC curve equals 0,5418.

Semen odour. The semen odour has not yet been proven to be related to any pathological condition in semen.

Semen colour. The relation between the semen colour and the presence of a microbe in the semen sample is not proven to be statistically important. However, the relation between the semen colour and the population of the spermatozoa is proven to be statistically important. Indeed, it is proven to be 24,03 times more probable for less opaque samples to contain a small population of spermatozoa and this result is proven to be statistically significant (p-value<0,001).

At first, it was checked if the semen colour in itself could predict whether the population of the spermatozoa is inside or outside the physiological values (Table 3.5).

Table 3.5. Sorting between the semen colour and the population of spermatozoa

colour	Population		Total
	-	+	
-	592	505	1097
+	4	82	86
Total	596	587	1183

It was observed that in 95,35% of the samples where the semen colour was less opaque than normal, the population of the spermatozoa was outside the physiological values (PPV). However, in 53,97% of the samples where the semen colour was normal, the population of the spermatozoa was inside the physiological values (NPV).

It was also checked whether the population of the spermatozoa could predict the semen colour.

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It was observed that in 13,97% of the samples where the population of the spermatozoa was low, the semen was less opaque than normal (sensitivity test). However, in 99,33% of the samples where the population of the spermatozoa was normal, the semen colour was normal (specificity test).

The accuracy of a test using the semen colour to distinguish between the samples where the population of the spermatozoa is low and those with a population of spermatozoa inside the physiological values is 56,97%, which corresponds to the percentage of the correctly classified samples in table 3.5. The area under the ROC curve equals 0,7466.

Sum of the physicochemical characteristics. The relation between the physicochemical characteristics of semen and the presence of a microbe in the semen sample is proven to be statistically important, exception made for the semen colour. Indeed, it is proven to be 1,90 times more probable to detect a microbe in a sample if at least one of its physicochemical characteristics, exception made for the semen colour, is outside the physiological values compared to those where these physicochemical characteristics are inside the physiological values and this result is proven to be statistically significant (p-value=0,019). In the following, the physicochemical characteristics do not include the semen colour.

At first, it was checked whether the presence of at least one of the physicochemical characteristics of semen being outside the physiological values could predict the detection of a microbe in the sample (Table 3.6).

Table 3.6. Sorting between the semen physicochemical characteristics and the presence of a microbe

Phys./chem.	Microb. exam		Total
	-	+	
-	19	73	92
+	133	972	1105
Total	152	1045	1197

It was observed that in 87,96% of the samples where at least one of the physicochemical characteristics of semen was outside the physiological values, a microbe was detected (PPV). However, in 20,65% of the samples where all the physicochemical characteristics of semen were inside the physiological values, no microbe was detected (NPV).

It was also checked if the detection of a microbe in the sample could predict whether at least one of the physicochemical characteristics of semen is outside the physiological values.

It was observed that in 93,01% of the samples where a microbe was detected, at least one of the physicochemical characteristics of semen was outside the physiological values (sensitivity test). However, in 12,50% of the samples where no microbe was detected, all the physicochemical characteristics of semen were inside the physiological values (specificity test).

The accuracy of a test using all the physicochemical characteristics of semen to distinguish between the infected samples and those without a microbe is 82,79%, which corresponds to the percentage of the correctly classified samples in table 3.6. The area under the ROC curve equals 0,5431.

3.3 Population of spermatozoa

The relation between the population of spermatozoa and the presence of a microbe in the semen sample is proven to be statistically important. Indeed, it is proven to be 1,89 times more probable to detect a microbe when the population of spermatozoa is lower than 98million and this result is proven to be statistically important (p-value<0,001).

At first, it was checked whether the population of spermatozoa in itself could predict the detection of a microbe in the sample (Table 3.7).

Table 3.7. Sorting between the population of spermatozoa and the presence of a microbe

Population	Microb. exam		Total
	-	+	
-	97	505	602
+	55	540	595
Total	152	1045	1197

It was observed that in 90,76% of the samples where the population of spermatozoa was outside the physiological values, a microbe was detected (PPV). However, in 16,11% of the samples where the population of spermatozoa was inside the physiological values, no microbe was detected (NPV).

It was also checked if the detection of a microbe in the sample could predict whether the population of spermatozoa is inside or outside the physiological values.

It was observed that in 51,67% of the samples where a microbe was detected, the population of spermatozoa was outside the physiological values (sensitivity test). However, in 63,82% of the samples where no microbe was detected, the population of spermatozoa was inside the physiological values (specificity test).

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The accuracy of a test using the population of spermatozoa to distinguish between the infected samples and those without a microbe is 53,22%, which corresponds to the percentage of the correctly classified samples in table 3.7. The area under the ROC curve equals 0,5343.

3.4 Motility assessment

The relation between the motility of spermatozoa and the presence of a microbe in the semen sample is proven to be statistically important. Indeed, it is proven to be 1,95 times more probable to detect a microbe if the progressive motility percentage is lower than 53,6% and this result is proven to be statistically important (p-value<0,001).

At first, it was checked whether the percentage of the spermatozoa presenting progressive motility in itself could predict the detection of a microbe in the sample (Table 3.8).

Table 3.8. Sorting between the progressive motility percentage and the presence of a microbe

Type (a+b)%	Microb. exam		Total
	-	+	
-	87	426	513
+	65	619	684
Total	152	1045	1197

It was observed that in 90,50% of the samples where the progressive motility percentage was outside the physiological values, a microbe was detected (PPV). However, in 16,96% of the samples where the progressive motility percentage was inside the physiological values, no microbe was detected (NPV).

It was also checked if the detection of a microbe in the sample could predict whether the progressive motility percentage is inside or outside the physiological values.

It was observed that in 59,23% of the samples where a microbe was detected, the progressive motility percentage was outside the physiological values (sensitivity test). However, in 57,24% of the samples where no microbe was detected, the progressive motility percentage was inside the physiological values (specificity test).

The accuracy of a test using the progressive motility percentage to distinguish between the infected samples and those without a microbe is 58,98%, which corresponds to the percentage of the correctly classified samples in table 3.8. The area under the ROC curve equals 0,5373.

3.5 Morphology assessment

Percentage of morphologically normal spermatozoa. The relation between the percentage of spermatozoa presenting normal morphology and the presence of a microbe in the semen sample is proven to be statistically important. Indeed, it is proven to be 2,02 times more probable to detect a microbe if the percentage of spermatozoa presenting normal morphology is lower than 4% and this result is proven to be statistically important (p-value<0,001).

At first, it was checked whether the percentage of the spermatozoa presenting normal morphology in itself could predict the detection of a microbe in the sample (Table 3.9).

Table 3.9. Sorting between the normal morphology percentage and the presence of a microbe

Normal %	Microb. exam		Total
	-	+	
-	85	393	478
+	57	533	590
Total	142	926	1068

It was observed that in 90,34% of the samples where the normal morphology percentage was outside the physiological values, a microbe was detected (PPV). However, in 17,78% of the samples where the normal morphology percentage was inside the physiological values, no microbe was detected (NPV).

It was also checked if the detection of a microbe in the sample could predict whether the normal morphology percentage is inside or outside the physiological values.

It was observed that in 57,56% of the samples where a microbe was detected, the normal morphology percentage was outside the physiological values (sensitivity test). However, in 59,86% of the samples where no microbe was detected, the normal morphology percentage was inside the physiological values (specificity test).

The accuracy of a test using the normal morphology percentage to distinguish between the infected samples and those without a microbe is 57,87%, which corresponds to the percentage of the correctly classified samples in table 3.9. The area under the ROC curve equals 0,5406.

Quantity of morphological abnormalities per spermatozoon. The relation between the Sperm Deformity Index (SDI) and the presence of a microbe in the semen sample is proven to be statistically important. Indeed, it is proven to be 2,03 times more probable to detect a microbe if the SDI is lower than 1,33 compared to samples with SDI≤1,33 and this result is proven to be statistically important (p-value<0,001).

At first, it was checked whether the SDI in itself could predict the detection of a microbe in the sample (Table 3.10).

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Table 3.10. Sorting between the SDI and the presence of a microbe

SDI	Microb. exam		Total
	-	+	
-	75	329	404
+	67	597	664
Total	142	926	1068

It was observed that in 89,91% of the samples where the SDI was outside the physiological values, a microbe was detected (PPV). However, in 18,56% of the samples where the SDI was inside the physiological values, no microbe was detected (NPV).

It was also checked if the detection of a microbe in the sample could predict whether the SDI is inside or outside the physiological values.

It was observed that in 64,47% of the samples where a microbe was detected, the SDI was outside the physiological values (sensitivity test). However, in 52,82% of the samples where no microbe was detected, the SDI was inside the physiological values (specificity test).

The accuracy of a test using the SDI to distinguish between the infected samples and those without a microbe is 62,92%, which corresponds to the percentage of the correctly classified samples in table 3.10. The area under the ROC curve equals 0,5424.

Kind of morphological abnormalities.

Head abnormalities[1]: The relation between the head abnormalities percentage and the presence of a microbe in the semen sample is proven to be statistically important. Indeed, it is proven to be 1,95 times more probable to detect a microbe if the percentage of head abnormalities is higher than 92% compared to samples with this percentage being ≤92% and this result is proven to be statistically important (p-value<0,001).

At first, it was checked whether the head abnormalities percentage in itself could predict the detection of a microbe in the sample (Table 3.11).

Table 3.11. Sorting between the head abnormalities percentage and the presence of a microbe

Head abn.	Microb. exam		Total
	-	+	
-	67	291	358
+	75	635	710
Total	142	926	1068

It was observed that in 89,44% of the samples where the percentage of the spermatozoa having abnormal head morphology was outside the physiological values, a microbe was detected (PPV). However, in 18,72% of the samples where the percentage of head abnormalities was inside the physiological values, no microbe was detected (NPV).

It was also checked whether the detection of a microbe in the sample could predict whether the percentage of head abnormalities is inside or outside the physiological values.

It was observed that in 68,57% of the samples where a microbe was detected, the percentage of head abnormalities was outside the physiological values (sensitivity test). However, in 47,18% of the samples where no microbe was detected, the head abnormalities percentage was inside the physiological values (specificity test).

The accuracy of a test using the percentage of head abnormalities to distinguish between the infected samples and those without a microbe is 65,73%, which corresponds to the percentage of the correctly classified samples in table 3.11. The area under the ROC curve equals 0,5408.

Big head: The relation between the percentage of spermatozoa having big head and the presence of a microbe in the semen sample is not proven to be statistically important. However, the big heads are strongly related to abnormalities of the midpiece, which are related to the presence of a microbe in the semen sample, in their turn.

Small head: The relation between the percentage of spermatozoa having small head and the presence of a microbe in the semen sample is proven to be statistically important. Indeed, it is proven to be 1,79 times more probable to detect a microbe if the percentage of small head is higher than 21% compared to samples with this percentage being ≤21% and this result is proven to be statistically important (p-value=0,002).

At first, it was checked whether a high percentage of spermatozoa having small head in itself could predict the detection of a microbe in the sample (Table 3.12).

Table 3.12. Sorting between the percentage of small head and the presence of a microbe

Small head	Microb. exam		Total
	-	+	
-	86	428	514
+	56	498	554
Total	142	926	1068

It was observed that in 89,89% of the samples where the percentage of small head was high, a microbe was detected (PPV). However, in 16,73% of the samples where the percentage of small head was inside the physiological values, no microbe was detected (NPV).

[1] The abnormalities of the acrosome were included in the calculation of this percentage.

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It was also checked if the detection of a microbe in the sample could predict whether the percentage of small head is inside or outside the physiological values.

It was observed that in 53,78% of the samples where a microbe was detected, the percentage of small head was high (sensitivity test). However, in 60,56% of the samples where no microbe was detected, the percentage of small head was inside the physiological values (specificity test).

The accuracy of a test using the percentage of small head to distinguish between the infected samples and those without a microbe is 54,68%, which corresponds to the percentage of the correctly classified samples in table 3.12. The area under the ROC curve equals 0,5331.

Round head: The relation between the percentage of spermatozoa having round head and the presence of a microbe in the semen sample is not proven to be statistically important.

Tapered head[2]: The relation between the percentage of spermatozoa having pyriform or tapered or narrow (ptn) head and the presence of a microbe in the semen sample is not proven to be statistically important. However, the relation between the percentage of spermatozoa having ptn head and the presence of *Ureaplasma u*. or *Mycoplasma h*. or *Chlamydia tr.* (umc) in the semen sample is proven to be statistically important. Indeed, it is proven to be 1,49 times more probable to detect umc if the percentage of ptn head is higher than 36% compared to samples with this percentage being ≤36% and this result is proven to be statistically important (p-value=0,002).

At first, it was checked whether a high percentage of spermatozoa having ptn head, i.e. the sum of the percentage of each one of them in itself could predict the detection of umc in the sample (Table 3.13).

Table 3.13. Sorting between the percentage of ptn head and the presence of umc

Ptn head	Umc		Total
	-	+	
-	266	368	634
+	142	292	434
Total	408	660	1068

It was observed that in 67,28% of the samples where the percentage of ptn head was high, umc was detected (PPV). However, in 41,96% of the samples where the percentage of ptn head was inside the physiological values, no microbe was detected (NPV).

It was also checked whether the detection of umc in the sample could predict whether the percentage of ptn head is inside or outside the physiological values.

It was observed that in 44,24% of the samples where umc was detected, the percentage of ptn head was high (sensitivity test). However, in 65,20% of the samples where umc was not detected, the percentage of ptn head was inside the physiological values (specificity test).

The accuracy of a test using the percentage of ptn head to distinguish between the infected samples and those without a microbe is 52,25%, which corresponds to the percentage of the correctly classified samples in table 3.13. The area under the ROC curve equals 0,5462.

Thin head[3]: The relation between the percentage of spermatozoa having notably pyriform or notably tapered or notably narrow (nptn) head and the presence of a microbe in the semen sample is proven to be statistically important. Interestingly, the relation between the percentage of spermatozoa having nptn head and the presence of umc in the semen sample is proven to be statistically important, too. Indeed, it is proven to be 2,11 times more probable to detect a microbe and 1,38 times more probable to detect umc in a semen sample if the percentage of nptn head is higher than 7% compared to samples with this percentage being ≤7% and this result is proven to be statistically important (p-value=0,002 for any microbe and p-value=0,010 for umc).

At first, it was checked whether a high percentage of spermatozoa having nptn head, i.e. the sum of the percentage of each one of them in itself could predict the detection of a microbe in the semen sample (Table 3.14).

Table 3.14. Sorting between the nptn head and the presence of a microbe

Nptn	Microb. exam		Total
	-	+	
-	85	383	468
+	57	543	600
Total	142	926	1068

It was observed that in 90,50% of the samples where the percentage of nptn head was high, a microbe was detected (PPV). However, in 18,16% of the samples where the percentage of nptn head was inside the physiological values, no microbe was detected (NPV).

It was also checked if the detection of a microbe in the sample could predict whether the

[2] These abnormalities included the pyriform and narrow shapes.

[3] These abnormalities are referred to the notably narrow, notably pyriform and notably tapered shapes.

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percentage of nptn head is inside or outside the physiological values.

It was observed that in 58,64% of the samples where a microbe was detected, the percentage of nptn head was high (sensitivity test). However, in 59,86% of the samples where no microbe was detected, the percentage of nptn head was inside the physiological values (specificity test).

The accuracy of a test using the percentage of nptn head to distinguish between the infected samples and those without a microbe is 58,80%, which corresponds to the percentage of the correctly classified samples in table 3.14. The area under the ROC curve equals 0,5433.

Regarding the relation between the percentage of spermatozoa having nptn head and the presence of umc, it was checked whether a high percentage of spermatozoa having nptn head, i.e. the sum of the percentage of each one of them in itself could predict the detection of umc in the semen sample (Table 3.15).

Table 3.15. Sorting between the nptn head and the presence of umc

Nptn	Umc		Total
	-	+	
-	199	269	468
+	209	391	600
Total	408	660	1068

It was observed that in 65,17% of the samples where the percentage of nptn head was high, umc was detected (PPV). However, in 42,52% of the samples where the percentage of nptn head was inside the physiological values, umc was not detected (NPV).

It was also checked whether the detection of umc in the sample could predict whether the percentage of nptn head is inside or outside the physiological values.

It was observed that in 59,24% of the samples where umc was detected, the percentage of nptn head was high (sensitivity test). However, in 48,77% of the samples where umc was not detected, the percentage of nptn head was inside the physiological values (specificity test).

The accuracy of a test using the percentage of nptn head to distinguish between the samples infected from umc and those without umc is 55,24%, which corresponds to the percentage of the correctly classified samples in table 3.15. The area under the ROC curve equals 0,5384.

Amorphous head: The relation between the percentage of spermatozoa having amorphous head and the presence of a microbe in the semen sample is not proven to be statistically important. However, the amorphous heads are strongly related to low percentage of progressive motility, abnormal acrosome, especially its absence, significant abnormalities of the midpiece and presence of cytoplasmic residuals, which are related to the presence of a microbe in the semen sample, in their turn.

Acrosome abnormalities[4]: The relation between the percentage of spermatozoa having an abnormal acrosome and the presence of a microbe in the semen sample is proven to be statistically important. Indeed, it is proven to be 2,06 times more probable to detect a microbe in a semen sample if the percentage of the acrosome abnormalities is higher than 81% compared to samples with this percentage being ≤81% and this result is proven to be statistically important (p-value<0,001).

At first, it was checked whether a high percentage of the acrosome abnormalities in itself could predict the detection of a microbe in the semen sample (Table 3.16).

Table 3.16. Sorting between the acrosome abnormalities and the presence of a microbe

Acrosome	Microb. exam		Total
	-	+	
-	78	344	422
+	64	582	646
Total	142	926	1068

It was observed that in 90,09% of the samples where the percentage of acrosome abnormalities was high, a microbe was detected (PPV). However, in 18,48% of the samples where the percentage of acrosome abnormalities was inside the physiological values, no microbe was detected (NPV).

It was also checked if the detection of a microbe in the sample could predict whether the percentage of acrosome abnormalities is inside or outside the physiological values.

It was observed that in 62,85% of the samples where a microbe was detected, the percentage of acrosome abnormalities was high (sensitivity test). However, in 54,93% of the samples where no microbe was detected, the percentage of acrosome abnormalities was inside the physiological values (specificity test).

The accuracy of a test using the percentage of acrosome abnormalities to distinguish between the infected samples and those without a microbe is 61,80%, which corresponds to the percentage of the correctly classified samples in table 3.16. The area under the ROC curve equals 0,5429.

[4] These abnormalities included the small, smaller or vacuolated acrosome distinguished later (after the development of the andro-test).

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Small acrosome: The relation between the small acrosome being a prevalent abnormality and the presence of a microbe in the semen sample is proven to be statistically important. Interestingly, the relation between the small acrosome being a prevalent abnormality and the presence of umc in the semen sample is proven to be statistically important, too. Indeed, it is proven to be 2,03 times more probable to detect a microbe and 1,46 times more probable to detect umc in a semen sample if the small acrosome is a prevalent abnormality compared to samples where this abnormality is not prevalent and this result is proven to be statistically important (p-value=0,007 for any microbe and p-value=0,018 for umc).

At first, it was checked whether the small acrosome being a prevalent abnormality in itself could predict the detection of a microbe in the semen sample (Table 3.17).

Table 3.17. Sorting between the small acrosome and the presence of a microbe

Small acros.	Microb. exam -	Microb. exam +	Total
-	124	715	839
+	18	211	229
Total	142	926	1068

It was observed that in 92,14% of the samples where the small acrosome was a prevalent abnormality, a microbe was detected (PPV). However, in 14,78% of the samples where the small acrosome was not a prevalent abnormality, no microbe was detected (NPV).

It was also checked if the detection of a microbe in the sample could predict whether the small acrosome is a prevalent abnormality.

It was observed that in 22,79% of the samples where a microbe was detected, the small acrosome was a prevalent abnormality (sensitivity test). However, in 87,32% of the samples where no microbe was detected, the small acrosome was not a prevalent abnormality (specificity test).

The accuracy of a test using the small acrosome being a prevalent abnormality to distinguish between the infected samples and those without a microbe is 31,37%, which corresponds to the percentage of the correctly classified samples in table 3.17. The area under the ROC curve equals 0,5346.

Regarding the relation between the small acrosome being a prevalent abnormality and the presence of umc, it was checked whether the small acrosome being a prevalent abnormality in itself could predict the detection of umc in the semen sample (Table 3.18).

It was observed that in 68,56% of the samples where the small acrosome was a prevalent abnormality, umc was detected (PPV). However, in 40,05% of the samples where the small acrosome was not a prevalent abnormality, umc was not detected (NPV).

Table 3.18. Sorting between the small acrosome and the presence of umc

Small across.	Umc -	Umc +	Total
-	336	503	839
+	72	157	229
Total	408	660	1068

It was also checked if the detection of umc in the sample could predict whether the small acrosome is a prevalent abnormality.

It was observed that in only 23,79% of the samples where umc was detected, the small acrosome was a prevalent abnormality (sensitivity test). However, in 82,35% of the samples where umc was not detected, the small acrosome was not a prevalent abnormality (specificity test).

The accuracy of a test using the small acrosome being a prevalent abnormality to distinguish between the samples infected from umc and those without umc is 46,16%, which corresponds to the percentage of the correctly classified samples in table 3.18. The area under the ROC curve equals 0,5430.

Absent acrosome: The relation between the absence of acrosome being a prevalent abnormality and the presence of a microbe in the semen sample is proven to be statistically important. Interestingly, the relation between the absence of acrosome being a prevalent abnormality and the presence of *Chlamydia tr.* in the semen sample is proven to be statistically important, too. Indeed, it is proven to be 4,00 times more probable to detect a microbe and 1,65 times more probable to detect *Chlamydia tr.* in a semen sample if the absence of acrosome is a prevalent abnormality, compared to samples where this abnormality is not prevalent and this result is proven to be statistically important (p-value<0,001 for any microbe and p-value=0,005 for *Chlamydia tr.*).

At first, it was checked whether the absence of acrosome being a prevalent abnormality in itself could predict the detection of a microbe in the semen sample (Table 3.19).

Table 3.19. Sorting between the absence of acrosome and the presence of a microbe

Absent acros.	Microb. exam -	Microb. exam +	Total
-	135	767	902
+	7	159	166
Total	142	926	1068

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It was observed that in 95,78% of the samples where the absence of acrosome was a prevalent abnormality, a microbe was detected (PPV). However, in 14,97% of the samples where the absence of acrosome was not a prevalent abnormality, no microbe was detected (NPV).

It was also checked if the detection of a microbe in the sample could predict whether the absence of acrosome is a prevalent abnormality.

It was observed that in 17,17% of the samples where a microbe was detected, the absence of acrosome was a prevalent abnormality (sensitivity test). However, in 95,07% of the samples where no microbe was detected, the absence of acrosome was not a prevalent abnormality (specificity test).

The accuracy of a test using the absence of acrosome being a prevalent abnormality to distinguish between the infected samples and those without a microbe is 27,53%, which corresponds to the percentage of the correctly classified samples in table 3.19. The area under the ROC curve equals 0,5537.

Regarding the relation between the absence of acrosome being a prevalent abnormality and the presence of *Chlamydia tr.*, it was checked whether the absence of acrosome being a prevalent abnormality in itself could predict the detection of *Chlamydia tr.* in the semen sample (Table 3.20).

Table 3.20. Sorting between the absence of acrosome and the presence of *Chlamydia tr.*

Absent acros.	*Chlamydia tr.* -	*Chlamydia tr.* +	Total
-	667	235	902
+	105	61	166
Total	772	296	1068

It was observed that in only 36,75% of the samples where the absence of acrosome was a prevalent abnormality, *Chlamydia tr.* was detected (PPV). However, in 73,95% of the samples where the absence of acrosome was not a prevalent abnormality, *Chlamydia tr.* was not detected (NPV).

It was also checked whether the detection of *Chlamydia tr.* in the sample could predict whether the absence of acrosome is a prevalent abnormality.

It was observed that in only 20,61% of the samples where *Chlamydia tr.* was detected, the absence of acrosome was a prevalent abnormality (sensitivity test). However, in 86,40% of the samples where *Chlamydia tr.* was not detected, the absence of acrosome was not a prevalent abnormality (specificity test).

The accuracy of a test using the absence of acrosome being a prevalent abnormality to distinguish between the samples infected from *Chlamydia tr*. infected samples and those without *Chlamydia tr.* is 68,16%, which corresponds to the percentage of the correctly classified samples in table 3.20. The area under the ROC curve equals 0,5535.

Midpiece abnormalities[5]: The relation between the percentage of midpiece abnormalities and the presence of a microbe in the semen sample is proven to be statistically important. Interestingly, the relation between the midpiece abnormalities and the presence of umc in the semen sample is proven to be statistically important, too. Indeed, it is proven to be 1,77 times more probable to detect a microbe and 1,59 times more probable to detect umc in a semen sample if the percentage of midpiece abnormalities is higher than 32% compared to samples where this percentage is lower or equal to 32% and this result is proven to be statistically important (p-value=0,002 for any microbe and p-value<0,001 for umc).

At first, it was checked whether the midpiece abnormalities in themselves could predict the detection of a microbe in the semen sample (Table 3.21).

Table 3.21. Sorting between the midpiece abnormalities and the presence of a microbe

Midpiece abn.	Microb. exam -	Microb. exam +	Total
-	68	316	384
+	74	610	684
Total	142	926	1068

It was observed that in 89,18% of the samples where the percentage of midpiece abnormalities was high, a microbe was detected (PPV). However, in 17,71% of the samples where the percentage of midpiece abnormalities was normal, no microbe was detected (NPV).

It was also checked if the detection of a microbe in the sample could predict whether the percentage of midpiece abnormalities is high.

It was observed that in 65,87% of the samples where a microbe was detected, the percentage of midpiece abnormalities was high (sensitivity test). However, in 47,89% of the samples where no microbe was detected, the percentage of midpiece abnormalities was normal (specificity test).

The accuracy of a test using the percentage of midpiece abnormalities to distinguish between the infected samples and those without a microbe

[5] These abnormalities did not include the cytoplasmic residuals.

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is 63,48%, which corresponds to the percentage of the correctly classified samples in table 3.21. The area under the ROC curve equals 0,5344.

Regarding the relation between the midpiece abnormalities and the presence of umc, it was checked whether the midpiece abnormalities in themselves could predict the detection of umc in the semen sample (Table 3.22).

Table 3.22. Sorting between the midpiece abnormalities and the presence of umc

Midpiece abn.	umc		Total
	-	+	
-	174	210	384
+	234	450	684
Total	408	660	1068

It was observed that in 65,79% of the samples where the percentage of midpiece abnormalities was high, umc was detected (PPV). However, in 45,31% of the samples where the percentage of midpiece abnormalities was normal, umc was not detected (NPV).

It was also checked if the detection of umc in the sample could predict whether the percentage of midpiece abnormalities is high.

It was observed that in 68,18% of the samples where umc was detected, the percentage of midpiece abnormalities was high (sensitivity test). However, in only 42,65% of the samples where umc was not detected, the percentage of midpiece abnormalities was normal (specificity test).

The accuracy of a test using the percentage of midpiece abnormalities to distinguish between the samples infected from umc and those without umc is 58,43%, which corresponds to the percentage of the correctly classified samples in table 3.22. The area under the ROC curve equals 0,5555.

Midpiece size abnormalities: The relation between the midpiece size abnormalities and the presence of a microbe in the semen sample is proven to be statistically important. Interestingly, the relation between the midpiece size abnormalities and the presence of umc in the semen sample is proven to be statistically important, too. Indeed, it is proven to be 1,67 times more probable to detect a microbe and 1,66 times more probable to detect umc in a semen sample if the percentage of midpiece size abnormalities is higher than 30% compared to samples where this percentage is lower or equal to 30% and this result is proven to be statistically important (p-value=0,005 for any microbe and p-value<0,001 for umc).

At first, it was checked whether the midpiece size abnormalities in themselves could predict the detection of a microbe in the semen sample (Table 3.23).

Table 3.23. Sorting between the midpiece size abnormalities and the presence of a microbe

Midpiece size abn.	Microb. Exam		Total
	-	+	
-	72	353	425
+	70	573	643
Total	142	926	1068

It was observed that in 89,11% of the samples where the percentage of the midpiece size abnormalities was high, a microbe was detected (PPV). However, in 16,94% of the samples where the percentage of the midpiece size abnormalities was normal, no microbe was detected (NPV).

It was also checked if the detection of a microbe in the sample could predict whether the percentage of the midpiece size abnormalities is high.

It was observed that in 61,88% of the samples where a microbe was detected, the percentage of the midpiece size abnormalities was high (sensitivity test). However, in 50,70% of the samples where no microbe was detected, the percentage of the midpiece size abnormalities was normal (specificity test).

The accuracy of a test using the percentage of the midpiece size abnormalities to distinguish between the infected samples and those without a microbe is 60,39%, which corresponds to the percentage of the correctly classified samples in table 3.23. The area under the ROC curve equals 0,5303.

Regarding the relation between the midpiece size abnormalities and the presence of umc, it was checked whether the midpiece size abnormalities in themselves could predict the detection of umc in the semen sample (Table 3.24).

Table 3.24. Sorting between the midpiece size abnormalities and the presence of umc

Midpiece size abn.	Umc		Total
	-	+	
-	193	232	425
+	215	428	643
Total	408	660	1068

It was observed that in 66,56% of the samples where the percentage of the midpiece size abnormalities was high, umc was detected (PPV). However, in 45,41% of the samples where the percentage of the midpiece size abnormalities was normal, umc was not detected (NPV).

It was also checked if the detection of umc in the sample could predict whether the percentage of the midpiece size abnormalities is high.

It was observed that in 64,85% of the samples where umc was detected, the midpiece

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size abnormalities percentage was high (sensitivity test). However, in only 47,30% of the samples where umc was not detected, the percentage of the midpiece size abnormalities was normal (specificity test).

The accuracy of a test using the percentage of the midpiece size abnormalities to distinguish between the samples infected from umc and those without umc is 58,15%, which corresponds to the percentage of the correctly classified samples in table 3.24. The area under the ROC curve equals 0,5599.

Cytoplasmic residuals: The relation between the cytoplasmic residuals and the presence of a microbe in the semen sample is proven to be statistically important. Interestingly, the relation between the cytoplasmic residuals and the presence of umc in the semen sample is proven to be statistically important, too. Indeed, it is proven to be 1,70 times more probable to detect a microbe and 1,59 times more probable to detect umc in a semen sample if the percentage of cytoplasmic residuals is higher than 4% compared to samples where this percentage is lower or equal to 4% and this result is proven to be statistically important (p-value=0,016 for any microbe and p-value=0,001 for umc).

At first, it was checked whether the cytoplasmic residuals in themselves could predict the detection of a microbe in the semen sample (Table 3.25).

Table 3.25. Sorting between the cytoplasmic residuals and the presence of a microbe

Cytopl. resid.	Microb. exam -	Microb. exam +	Total
-	113	645	758
+	29	281	310
Total	142	926	1068

It was observed that in 90,65% of the samples where the cytoplasmic residuals percentage was high, a microbe was detected (PPV). However, in 14,91% of the samples where the cytoplasmic residuals percentage was normal, no microbe was detected (NPV).

It was also checked whether the detection of a microbe in the sample could predict whether the cytoplasmic residuals percentage is high.

It was observed that in only 30,35% of the samples where a microbe was detected, the cytoplasmic residuals percentage was high (sensitivity test). However, in 79,58% of the samples where no microbe was detected, the cytoplasmic residuals percentage was normal (specificity test).

The accuracy of a test using the cytoplasmic residuals percentage to distinguish between the infected samples and those without a microbe is 36,89%, which corresponds to the percentage of the correctly classified samples in table 3.25. The area under the ROC curve equals 0,5278.

Regarding the relation between the cytoplasmic residuals and the presence of umc, it was checked whether the cytoplasmic residuals in themselves could predict the detection of umc in the semen sample (Table 3.26).

Table 3.26. Sorting between the cytoplasmic residuals and the presence of umc

Cytopl. resid.	Umc -	Umc +	Total
-	313	445	758
+	95	215	310
Total	408	660	1068

It was observed that in 69,35% of the samples where the cytoplasmic residuals percentage was high, umc was detected (PPV). However, in 41,29% of the samples where the cytoplasmic residuals percentage was normal, umc was not detected (NPV).

It was also checked whether the detection of umc in the sample could predict whether the cytoplasmic residuals percentage is high.

It was observed that in only 32,58% of the samples where umc was detected, the cytoplasmic residuals percentage was high (sensitivity test). However, in 76,72% of the samples where umc was not detected, the cytoplasmic residuals percentage was normal (specificity test).

The accuracy of a test using the cytoplasmic residuals percentage to distinguish between the samples infected from umc and those without umc is 49,44%, which corresponds to the percentage of the correctly classified samples in table 3.26. The area under the ROC curve equals 0,5532.

Significant abnormalities of the midpiece: The relation between the significant abnormalities of the midpiece being a prevalent abnormality and the presence of a microbe in the semen sample is proven to be statistically important. Interestingly, the relation between the significant abnormalities of the midpiece being a prevalent abnormality and the presence of umc in the semen sample is proven to be statistically important, too. Indeed, it is proven to be 4,32 times more probable to detect a microbe and 1,98 times more probable to detect umc in a semen sample if the significant abnormalities of the midpiece are a prevalent abnormality compared to samples where this kind of abnormalities is not a prevalent abnormality and this result is proven to be statistically important (p-value=0,002 for any microbe and p-value=0,001 for umc).

At first, it was checked whether the significant abnormalities of the midpiece being a prevalent abnormality in itself could predict the

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detection of a microbe in the semen sample (Table 3.27).

Table 3.27. Sorting between the significant abnormalities of the midpiece and the presence of a microbe

Signif. abn. midpiece	Microb. exam -	Microb. exam +	Total
-	137	800	937
+	5	126	131
Total	142	926	1068

It was observed that in 96,18% of the samples where the significant abnormalities of the midpiece were a prevalent abnormality, a microbe was detected (PPV). However, in 14,62% of the samples where the significant abnormalities of the midpiece were not a prevalent abnormality, no microbe was detected (NPV).

It was also checked if the detection of a microbe in the sample could predict whether the significant abnormalities of the midpiece are a prevalent abnormality.

It was observed that in only 13,61% of the samples where a microbe was detected, the significant abnormalities of the midpiece were a prevalent abnormality (sensitivity test). However, in 96,48% of the samples where no microbe was detected, the significant abnormalities of the midpiece were a prevalent abnormality (specificity test).

The accuracy of a test using the significant abnormalities of the midpiece being a prevalent abnormality to distinguish between the infected samples and those without a microbe is 24,63%, which corresponds to the percentage of the correctly classified samples in table 3.27. The area under the ROC curve equals 0,5504.

Regarding the relation between the significant abnormalities of the midpiece being a prevalent abnormality and the presence of umc, it was checked whether the significant abnormalities of the midpiece being a prevalent abnormality in itself could predict the detection of umc in the semen sample (Table 3.28).

Table 3.28. Sorting between the significant abnormalities of the midpiece and the presence of umc

Signif. abn. midpiece	Umc -	Umc +	Total
-	313	445	758
+	95	215	310
Total	408	660	1068

It was observed that in 74,81% of the samples where the significant abnormalities of the midpiece were a prevalent abnormality, umc was detected (PPV). However, in 40,02% of the samples where the significant abnormalities of the midpiece was not a prevalent abnormality, umc was not detected (NPV).

It was also checked whether the detection of umc in the sample could predict whether the significant abnormalities of the midpiece are a prevalent abnormality.

It was observed that in only 14,85% of the samples where umc was detected, the significant abnormalities of the midpiece were a prevalent abnormality (sensitivity test). However, in 91,91% of the samples where umc was not detected, the significant abnormalities of the midpiece were not a prevalent abnormality (specificity test).

The accuracy of a test using the significant abnormalities of the midpiece being a prevalent abnormality to distinguish between the samples infected from umc and those without umc is 44,29%, which corresponds to the percentage of the correctly classified samples in table 3.28. The area under the ROC curve equals 0,5338.

Tail abnormalities: The tail abnormalities have not yet been proven to be related to the presence of a microbe in the semen sample. However, if the percentage of tail abnormalities is higher than 4%, it is 1,80 times more probable (p-value<0,001) that the progressive motility is abnormal, too.

3.6 Semen Self-Exam

The Semen Self-Exam consists of the assessment of four semen characteristics that every man can assess in the comfort of his personal space, without any laboratory or specialized equipment. Three of these characteristics, the volume, the liquefaction and the viscosity, assess the health condition of the accessory glands (mainly the prostate and the seminiferous tubules) and their abnormal values are related to an infection of the genital tract (odds ratio=1,60 and p-value=0,040). The fourth characteristic, the semen colour, assesses mainly the function of the testicles and its abnormal values are related to low population of spermatozoa in the semen sample (odds ratio=24,03 and p-value<0,001).

At first, it was checked whether the Semen Self-Exam in itself could predict the detection of a microbe in the sample considering the first three characteristics (Table 3.29).

Table 3.29. Sorting between the Semen Self-Exam and the presence of a microbe

Semen Self-Exam	Microb. exam -	Microb. exam +	Total
-	28	129	157
+	124	916	1040
Total	152	1045	1197

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It was observed that in 88,08% of the samples where at least one of these three physicochemical characteristics of the Semen Self-Exam was outside the physiological values, a microbe was detected (PPV). However, in 17,83% of the samples where these characteristics were inside the physiological values, no microbe was detected (NPV).

It was also checked if the detection of a microbe in the sample could predict whether at least one of these physicochemical characteristics of the Semen Self-Exam is outside the physiological values.

It was observed that in 87,66% of the samples where a microbe was detected, at least one of these physicochemical characteristics of the Semen Self-Exam was outside the physiological values (sensitivity test). However, in 18,42% of the samples where no microbe was detected, these physicochemical characteristics of the Semen Self-Exam were inside the physiological values (specificity test).

The accuracy of a test using the previously mentioned physicochemical characteristics of the Semen Self-Exam to distinguish between the infected samples and those without a microbe is 78,86%, which corresponds to the percentage of the correctly classified samples in table 3.29. The area under the ROC curve equals 0,5296.

The relation between the semen colour and the population of spermatozoa was analyzed previously.

3.7 The semen analysis andro-test

At first, it was checked whether the andro-test in itself could predict the detection of a microbe in the sample (Table 3.30).

Table 3.30. Sorting between the andro-test and the presence of a microbe

andro-test	Microb. exam		Total
	-	+	
-	7	13	20
+	145	1032	1177
Total	152	1045	1197

It was observed that in 87,68% of the samples where at least one of the parameters of the andro-test was outside the physiological values, a microbe was detected (PPV). However, in 35,00% of the samples where all the parameters of the andro-test were inside the physiological values, no microbe was detected (NPV).

It was also checked if the detection of a microbe in the sample could predict whether at least one of the parameters of the andro-test is outside the physiological values.

It was observed that in 98,76% of the samples where a microbe was detected, at least one of the parameters of the andro-test was outside the physiological values (sensitivity test). However, in 4,61% of the samples where no microbe was detected, all the parameters of the andro-test were inside the physiological values (specificity test).

The accuracy of the andro-test to distinguish between the infected samples and those without a microbe is 86,80%, which corresponds to the percentage of the correctly classified samples in table 3.30. The area under the ROC curve equals 0,6134.

4. Concluding remarks

The semen parameters have been related to pathological conditions of the male genital tract and this relation has been proven to be statistically important. The present study evaluated the capability of each semen parameter to predict the presence of a pathological condition, mainly the presence of a microbe in the semen sample in exam (Table 3.31).

A urogenital tract infection in men first affects the physicochemical parameters of semen, since they describe the function of the prostate and the seminiferous tubules, which are placed in the front genital tract. This study confirmed that in the presence of a urogenital tract infection at least one of these semen parameters is outside the physiological values. This is why the less of these parameters are assessed the less probable it is to suspect the presence of a microbe in the semen sample in exam.

The capability of each semen parameter to predict the presence of a microbe in the semen sample in exam (PPV) is proven to be excellent. In most cases this capability exceeds 89%. Moreover, some of these parameters can satisfactorily predict the presence of umc in the sample in exam. It is not surprising that in some cases regardless of the fact that the semen parameter is outside the physiological values, no microbe is detected in the semen sample in exam. Part of these cases may represent those actually physiological semen samples, whose parameters present values outside the 95% confidence interval that determined the physiological values. Some cases may be related to the presence of another pathological condition, e.g. a varicocele. Some other cases may be related to a permanent damage in the genital tract due to the chronicity of an infection, which may still affect the semen parameters regardless of the fact that the infection is treated. Indeed, the infections in men usually remain untreated for a long time, because

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Table 3.31. Summarizing table of the odds ratio, the sensitivity, the specificity, the predictive value, the accuracy and the AUROC of each single parameter of semen and of specific sets of them.

	Test parameter	Event predicted	Odds Ratio	p-value	PPV	NPV	Sensitivity	Specificity	Accuracy	ROC area
Physicochemical characteristics	Volume	Infection	1,501	0,029	89,94	14,38	40,19	69,08	43,86	0,5464
	pH	Infection	2,003	<0,001	91,35	15,94	46,51	69,74	49,46	0,5812
	Liquefaction	Infection	1,457	0,034	89,48	14,62	48,04	61,18	49,71	0,5461
	Viscosity	Infection	1,953	0,050	89,15	19,21	44,57	70,83	48,69	0,5770
	Colour	Low population	24,032	<0,001	95,35	53,97	13,97	99,33	56,97	0,5665
	Physicochem.	Infection	1,902	0,019	87,96	20,65	93,01	12,50	82,79	0,5276
Population & Motility	Population	Infection	1,886	<0,001	90,76	16,11	51,67	63,82	53,22	0,5775
	type (a+b)	Infection	1,945	<0,001	90,50	16,96	59,23	57,24	58,98	0,5824
Morphology	Normal forms	Infection	2,023	<0,001	90,34	17,78	57,56	59,86	57,87	0,5871
	SDI	Infection	2,031	<0,001	89,91	18,56	64,47	52,82	62,92	0,5864
	Head abn.	Infection	1,949	<0,001	89,44	18,72	68,57	47,18	65,73	0,5788
	Small	Infection	1,787	0,002	89,89	16,73	53,78	60,56	54,68	0,5717
	Pyriform	Infection umc	1,486	0,002	67,28	41,96	44,24	65,20	52,25	0,5472
	Thin	Infection	2,114	<0,001	90,50	18,16	58,64	59,86	58,80	0,5925
	Thin	Infection umc	1,384	0,010	65,17	42,52	59,24	48,77	55,24	0,5401
	Acrosome	Infection	2,062	<0,001	90,09	18,48	62,85	54,93	61,80	0,5889
	Small acr.	Infection	2,033	0,007	92,14	14,78	22,79	87,32	31,37	0,5506
	Small acr.	Infection umc	1,457	0,018	68,56	40,05	23,79	82,35	46,16	0,5307
	No acr.	Infection	3,998	<0,001	95,78	14,97	17,17	95,07	27,53	0,5612
	No acr.	*Chlamydia tr.*	1,649	0,005	36,75	73,95	20,61	86,40	68,16	0,5350
	Midpiece (MP)	Infection	1,774	0,002	89,18	17,71	65,87	47,89	63,48	0,5688
	Midpiece (MP)	Infection umc	1,593	<0,001	65,79	45,31	68,18	42,65	58,43	0,5541
	Midpiece Size	Infection	1,670*	0,005*	89,11	16,94	61,88	50,70	60.39	0,5629
	Midpiece Size	Infection umc	1,656	<0,001	66,56	45,41	64,85	47,30	58,15	0,5608
	Cytopl. resid.	Infection	1,698*	0,016*	90,65	14,91	30,35	79,58	36,89	0,5496
	Cytopl. resid.	Infection umc	1,592	0,001	69,35	41,29	32,58	76,72	49,44	0,5465
	MP sign. abn.	Infection	4,316	0,002	96,18	14,62	13,61	96,48	24,63	0,5504
	MP sign. abn.	Infection umc	1,982	0,001	74,81	40,02	14,85	91,91	44,29	0,5338
Tests	Semen Self-Exam	Infection	1,603	0,040	88,08	17,83	87,66	18,42	78,86	0,5304
	andro-test	Infection	3,832	0,005	87,68	35,00	98,76	4,61	86,80	0,5168

* In https://doi.org/10.30551/ijs.v1i1.1 (Voulgaridis, 2018), in the table 8.3.2, these values are wrongly typed. The correct values are reported here.

the patients rarely complain about symptoms and men are not used to having a periodic andrological checkup.

On the other hand, the capability of each semen parameter to predict the absence of a microbe in the semen sample in exam (NPV) was already expected to be proven low, since: a) a urogenital tract infection does not always affect every organ, b) each organ may be affected in a different grade, and c) each patient's immunitary response may differ. Therefore, the accuracy (ACC) of diagnostic tests assessing just a single or a couple of semen parameters is expected to be poor.

Studying the specificity of each of the semen parameters it is noticed that the average between these specificities exceeds 65%. It is also noticed that the higher the number of samples being negative (inside the physiological values) to the parameter tested, the more the specificity percentage increases.

The Semen Self-Exam can accurately predict a low population of spermatozoa (PPV=95,35%) as much as the presence of a urogenital tract

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infection (PPV=88,08%), when at least one of its four parameters is outside the physiological values. However, it appears that it cannot exclude a pathological condition when all four of its parameters are inside the physiological values. This observation is due to a) the fact that some of the physicochemical parameters are not included in this self-exam because of the need of laboratory equipment, and b) the low percentage of these negative samples compared to the total (only 157 out of 1197).

A urogenital tract infection almost always (98,76%) affects at least one of the semen parameters of the andro-test, which appears to be outside the physiological values. Inversely, in almost 9 out of 10 cases (87,68%), if at least one of the semen parameters is outside the physiological values, a microbe is detected in the semen sample in exam. However, the specificity of the andro-test is low, regardless of the fact that 86,80% of the cases are correctly classified. The specificity of the andro-test appears to be low because of the very low percentage of samples in which the values of all the parameters of semen were inside the physiological values (only 20 out of 1197 samples).

As a result, although the andro-test cannot be considered a diagnostic test, since it provides us only with the indications that there is a pathological condition, it is proven to be an excellent screening test, which prompts for further investigation in the context of the Annual Andrological Checkup.

Unfortunately, the vast majority of the samples assessed in our study derive from men between 30 and 45 years old and the probability of having been infected several years ago was significant and unknown, since, up to nowadays, men had not been taught to preventively visit an urologist periodically since their adolescence. Future experimental studies on individuals with a known andrological history, where semen samples with all semen parameters inside the physiological values will undergo a complete microbiological exam, are expected to refine our results on the specificity and the NPV of the andro-test, thus, improving our knowledge in men's health.

5. Conflict of interest

The non-profit making non-governmental organization *Hellenic Association of Spermatology* (HAS) was constituted in 2017 with the purpose to promote knowledge, quality control and good practice in the field of human Spermatology in order to protect the human health. For the purpose of disseminating the knowledge acquired from the proper study of the human semen throughout the world, the non-profit open-access *International Journal of Spermatology* (IJS) was established in 2017. The author of the present study, owner of SpermLab, is the one who invented the andro-test, constituted HAS, established IJS and became its Editor-in-Chief. Inevitably, the editors of IJS have been aware of the conduction of the present study thus, reasonably motivating their judgment in the decision for the publication of the present study. The author declares that the fact that the present study is an observational and not an experimental one, proves that the assessment of the semen samples was not influenced by facts that happened later. Concluding, although the decision for publication could have been motivated, the data and the statistical analysis were not. Since our knowledge advances each time it is put into question, the author welcomes future studies that may refine the present findings.

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