Commentary

iMedPub Journals
www.imedpub.com

Annals of Clinical and Laboratory Research
ISSN 2386-5180

2021
Vol.9 No.12:387

DOI: 10.36648/2386-5180.9.12.387

Semen Parameters, Including WHO Strict Criteria in a Particular Region

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Received: November 05, 2021, **Accepted:** December 16, 2021, **Published:** December 23, 2021

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Citation: Kocturk SA (2021) Semen Parameters, Including WHO Strict Criteria in a Particular Region. Vol.9 No.12:387

Introduction

Male factor had been evaluated as responsible for approximately half of all infertility cases [1]. The initial assessment of the male patient involves a conventional Semen Analysis (SA), which may fail to provide a complete understanding of fertility potential due to variations in sperm quantity and quality.

In response to a growing need for the standardization of procedures for the examination of human semen; the WHO laboratory manual for the examination of human semen was first published in 1980. Until now, it has been updated five times and used extensively by research and clinical laboratories through the world. Despite this success, it has become apparent that some recommendations from previous editions of the manual needed to be revised in light of new evidence, even the editorial committee developed a consensus position after evaluating the pertinent literature. Therefore, we thought that our data collected according to these strict criteria may shed light to new editions.

We collected our data by performing our semen examination according to Kruger's criteria within WHO laboratory manual (2010). Initial semen analysis of a population admitted to urology clinic with infertility was done and the controversy over the significance of a cut off value defining fertile from unfertile men with knowledge of the clinical history. We assessed our patient about clinical history including varicocele, idiopathic infertility, recurrent miscarriages, ICSI failures cycles and exposure to environmental risk factors may lead to broken spermatogenesis so defective production of new spermatozoa; so we collected the results of the patients and correlated them to.

This article considers which men are most suitable for providing a reference population, presents data from such a population, mentions the possible limitations of the results obtained and discusses how the reference intervals could be interpreted as useful reference limits with socio demographic data. Obtaining clear reference ranges should help reduce the incidence of misdiagnosis of fertility problems and improve clinical care.

Study Population

Study approval was obtained from the Kahramanmaras Sutcu Imam University Medicine faculty ethical board. The study was conducted on 495 unselected patients with infertility lasting at least for 12 months admitted for sperm examination between January 2017-January 2021. We took into consideration about the patients' status including varicocele, idiopathic infertility, recurrent miscarriages, ICSI failures cycles, an incorrect lifestyle, and exposure to environmental risk factors.

A retrospective chart review from a retrospectively collected database at the Markasi Hospital Laboratory. Samples were reviewed over a 3-year period between September 2017 and September 2020. All semen samples were reviewed and only included morphology readings under both the WHO4 and WHO5 methods. Men with azoospermia and/or incomplete data were excluded.

Sample Collection

All individuals were provided instructions on sample collection, including collection after self-stimulation into a clean container. Samples were immediately provided to the laboratory, for processing by the microbiologist. Samples were prepared according to the WHO laboratory manual 5th edition.

Sperm morphology was characterized with two sets of criteria based on the WHO manual. First, the samples were assessed using the WHO4 edition, which included an assessment of sperm morphology based on normal-appearing heads, mid-pieces, and tails for which a cut off of 14% was employed. Second, the samples were then assessed using the WHO5 edition, which required a cut off of 4% and a strict morphometric assessment [2].

Method

Data on semen volume, sperm concentration, total sperm number per ejaculate, leucocyte number, pH, motility, vitality

and normal morphology were included only if they were generated from complete semen samples, obtained following 2-7 days of sexual abstinence. This range was used because this is the interval recommended by the WHO manual (2010) and it has thus become a standard practice.

We do semen analysis in our laboratory involving the following steps which are described in detail in the WHO manual (2010) [2]. In the first 5 minutes: Placing the specimen container on the bench or in an incubator (37 °C) for liquefaction. Between 30 and 60 minutes:

1. Assessing liquefaction and appearance of the semen. Measuring semen volume.
2. Measuring semen pH.
3. Preparing a wet preparation for assessing microscopic appearance, sperm motility and the dilution required for assessing sperm number.
4. Assessing sperm vitality (if the percentage of motile cells is low).
5. Making semen smears for assessing sperm morphology.
6. Making semen dilutions for assessing sperm concentration. Assessing sperm number.
7. Assessing peroxidase-positive cells (if round cells are present).
8. After 4 hours: Fixing, staining and assessing smears for sperm morphology. Later on the same day (or on a subsequent day if samples are frozen).

We identified cut-off values relating the number of semen pH, volume, alive spermatozoa, progressive motility, total motility and normal morphology. In morphologic assessment lower limit for abnormality was 4%. Upper values accepted as normal morphology. In pH assessment, we accepted the value of 7.2 as a lower threshold value. The lower reference limit for semen volume is 2 ml. Lower limit for alive spermatozoa (either motile or not) was 40%, as vitality test. The lower reference limit for number of sperm in semen was 20 million /ml. Abstinence of leucocytes was also one of the criteria.

We also assessed our patients about their clinical history including varicocele, idiopathic infertility, recurrent miscarriages, ICSI failures cycles, and exposure to environmental risk factors to correlate the statistical values we had.

The Mann-Whitney Test was used to assess the relationship of dichotomus variables with the parameters of the spermiogram. We used also Spearman correlation to analyze the relationship between age and the parameters.

Results and Discussion

We used spearman correlation test to analyze the relationship between age and the motility **(Table 1)** and Mann-whitney U test for analyzing relationship between age and other parameters **(Table 2).** Mann-Whitney Test showed also that the citizenship (codified as "Turkish/ Syrian") influences some parameters: pH, vitality, number of spermatozoa, sperm concentration, with worse results for the Syrian group. There is no statistically significant relation between the age and motility, there is no positive effect of young age was found on motility **(Table 1).** Moreover, no statistically significant relation was present with other parameters such as sperm concentration, presence of leucocytes in semen, lasted liquefaction time, morphology defects, vitality and regeneration capacity **(Table 2).**

Spearman correlation test

Mann Whitney U test

We found the progressive sperm motility was also decreasing with lower sperm counts as in previous studies. While progressive and nonprogressive motile sperm percentage decreases, nonmotile sperm percentage increases under the sperm values of less than 20 million per mL. Moreover, no statistically significant relation with existence of varicocele, gynecological problem in his partner, abortus history, IVF history, child existence, pH and volume of semen **(Table 3).**

Although head and midpiece abnormality percentages did not change between our groups, tail abnormalities inversely correlate with sperm count; therefore, percentage of sperm with tail abnormality directly affects the concentration. They detected deterioration of sperm morphology in all parts including tail, midpiece and head with suppressed sperm counts. On the contrary, we only detected significant relation with tail abnormalities. Therefore, we thought tail abnormalities seem to be more determining factor in low sperm counts **(Figure 1).**

Characteristics of the mean values of semen analysis of the group are shown in (**Table 4)**. There was no difference for head and midpiece abnormality among groups. However, a significant difference was detected between each group for the progressive sperm motility ($p<0.01$). Progressive sperm motility significantly correlated with normal morphology ($p<0.001$) **(Table 5).** A negative correlation was detected between nonmotile sperm count and normal morphology ($r=-373$, $p<0.001$). Among morphologic abnormalities, tail problems were more prominent relating to infertility problems in previous studies.

We reported the percentages of normal values of the group. We have seen that being an abnormal feature for infertility, partner's gynecological problem found to be 2.53% so less important. Moreover abortus history has been found to be 3%, IVF history was 1.01% in group. Previously 7.58% of the group has had a child.

Table 1: Age and motility relationship.

Progressive Motility		
Age	r	-0,067
	p	0,347

Table 2: Cases Selected For Alkaline Hemoglobin Electrophoresis On The Basis Of Discrimination Index / Indices.

		Age			p
		Mean	s.s.	Median	
Sperm concentration	<20 billion	30,03	± 6,40	30,00	0.676
	>20 billion	29,89	± 6,30	28,00	
Infection sign	No	29,97	± 5,38	28,00	0.264
	Yes	29,89	± 7,25	28,00	
Liquefaction time	Lasted >45 min	29,12	± 4,22	28,50	0.794
	<45 min	30,06	± 6,57	28,00	
Morphology	<%4	30,30	± 6,11	29,00	0.147
	>%4	29,58	± 6,51	27,00	
Vitality	Abnormal	30,51	± 6,65	30,00	0.271
	Normal	29,79	± 6,24	28,00	
Regeneration capacity (spermatogenesis)	No	30,38	± 6,70	29,50	0.494
	Yes	29,84	± 6,24	28,00	

Table 3: Mean values related to motility.

		Progressive motility			p	Total motility			p
		Med	s.s.	Median		Med	s.s.	Median	
Varicocele	yes	31,12	± 23,81	30,00	0,318	65,68	± 35,66	78,00	0,677
	no	34,21	± 21,97	31,50		70,26	± 31,79	83,00	
Gynecological disease in his partner	yes	32,34	± 23,11	31,00	0,555	67,42	± 34,31	81,00	0,762
	no	26,00	± 28,20	30,00		61,00	± 40,30	84,00	
Abortus history	no	31,90	± 23,10	30,50	0,332	66,89	± 34,37	80,50	0,294
	yes	41,17	± 26,39	40,50		79,00	± 35,43	92,00	
IVF	no	32,00	± 23,15	30,50	0,339	67,00	± 34,44	81,00	0,299
	yes	49,50	± 27,58	49,50		92,50	± 9,19	92,50	
Childbearing	no	31,50	± 23,15	30,00	0,176	66,68	± 35,09	81,00	0,867
	yes	40,47	± 22,80	34,00		74,33	± 23,61	85,00	
pH	abnormal	23,36	± 18,83	18,00	0,126	67,86	± 31,28	79,00	0,874
	Normal (between7,2-9)	32,85	± 23,39	32,00		67,21	± 34,67	81,00	
Volume	< 2ml	29,45	± 22,23	29,50	0,459	63,83	± 36,93	76,50	0,387
	>2 ml	32,87	± 23,44	30,50		68,13	± 33,76	81,50	
Sperm concentration	<20 billion	11,93	± 17,04	3,50	<0,001	29,83	± 30,42	25,50	<0,001
	>20 billion	40,98	± 19,76	42,00		83,53	± 20,50	92,00	

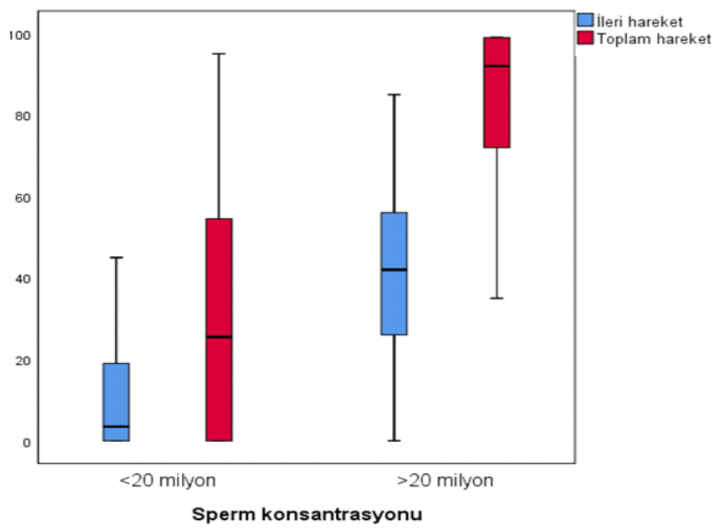

Figure 1 Graphic sperm concentration.

Table 4: Mean values.

		n
Age		29,93 ± 6,31
Varicocele	No	130
	Yes	68
Gynecological problem in his partner	No	193
	Yes	5
Abortus	No	192
	Yes	6
IVF history	No	196
	Yes	2
Child existence	No	183
	Yes	15
pH	abnormal	14
	Between 7,2-9 (normal)	184
Volume	≤ 2 ml	40
	>2 ml	158
Sperm concentration	<20 billion	60
	>20 billion	138
Infection-leucocyte	No	105
	Yes	93
Liquefaction time	Lasted more than 45 min	26
	≤ 45 min	172
Progressive motility		32,18 ± 23,19
Total motility		67,26 ± 34,37
Morphology(normal structure)	<%4	98
	≥ %4	100
Vitality	Not normal	39
	Normal	159
Regeneration capacity	No	34
	Yes	164

Table 5: Sperm concentration.

		Sperm concentration				p
		<20 million		>20 million		
		n	%	n	%	
Varikocele	No	42	(70,00)	88	(63,77)	0,396
	Yes	18	(30,00)	50	(36,23)	
Gynecological problem in his partner	No	58	(96,67)	135	(97,83)	0,633
	Yes	2	(3,33)	3	(2,17)	
Abortus history	no	59	(98,33)	133	(96,38)	0,460
	yes	1	(1,67)	5	(3,62)	
IVF history	no	60	(100,00)	136	(98,55)	0,349
	yes	0	(,00)	2	(1,45)	
Childbearing	no	57	(95,00)	126	(91,30)	0,366
	yes	3	(5,00)	12	(8,70)	
pH	abnormal	4	(6,67)	10	(7,25)	0,884
	Between 7,2-9 (normal)	56	(93,33)	128	(92,75)	
Volume	< 2ml	14	(23,33)	26	(18,84)	0,469
	>2ml	46	(76,67)	112	(81,16)	
Infection/leucocyte	no	39	(65,00)	66	(47,83)	**0,026**
	yes	21	(35,00)	72	(52,17)	
Liquefaction time	Lasted >45 min	15	(25,00)	11	(7,97)	**0,001**
	<45 min	45	(75,00)	127	(92,03)	
Morphology	<%4 normal spermatozoa	45	(75,00)	53	(38,41)	**<0,001**
	>%4 normal spermatozoa	15	(25,00)	85	(61,59)	

Vitality	< 40%	35	(58,33)	4	(2,90)	**<0,001**
	>40%	25	(41,67)	134	(97,10)	
Regeneration capacity	no	29	(48,33)	5	(3,62)	**<0,001**
	yes	31	(51,67)	133	(96,38)	

Discussion

For the past several decades, the World Health Organization (WHO) laboratory manual for the examination of human sperm has been the primary reference for methods of semen analysis. The only way that quantitative parameter terminology can be used is to state a value as ''above'' or ''below'' minimum reference values. The values created in the 2010 WHO study were from 4,500 fertile men. The WHO did not examine semen analyses from infertile men and therefore did not define men as infertile if they were below the one-sided 95% confidence interval of fertile men.

Despite our ability to assess sperm quality through a semen analysis methodology harmonized across laboratories, the use of these parameters cannot precisely and accurately predict the fertility of a man presenting to a clinician. This is because there are many factors in addition to sperm and semen quality that contribute to the ability of spermatozoa to fertilize an oocyte. For example; the age variable was used in the study as a b-spline to take into account a possible non-linear relation. The putative non-linear effects of these variables seem to change cut-offs. If researchers take into consideration, predictions for large groups of patients, prohibiting the possibility to give more accurate data. Similar results were obtained in the validation of tests. In our study we are evaluating total sperm count as fertility sign; a total number above 20 million/ml as concentration of spermatozoa had been classified as fertile. We found no meaningful relationship between sperm concentration and other parameters of patient such as; progressive motility, pH of semen, patient's previous history of IVF, childbearing, abortus any other gynaecological history. It is presented as being important, although clinically such a relationship is weak. The following conversation may take place; some other parameters influence the total sperm number per ejaculate [3]. There is argument that some other parameters affecting function such as testicular size [4].

In a different study; the reduction of sperm motility due to the decrease of the percentage of live spermatozoa is a physiological event [5]. Our study has shown that, when the percentage of live spermatozoa falls below 71.7%, sperm motility and sperm concentration are reduced. It has been reported that caspase enzymatic activity is higher in semen samples with low motility. The present study not only showed that LMMP correlated with sperm progressive, total motility, and volume, but also that it is possible to identify a threshold of 36.5% above which the probability of finding conventional sperm parameter abnormality increases. We also identified a threshold of HMMP of ≥ 46.25% (t).

Spermatogenesis occurs continuously. Each germ cell requires about 72 to 74 days maturing fully. Spermatogenesis is most efficient at 34° C. Within the seminiferous tubules, Sertoli cells regulate maturation, and Leydig cells produce the necessary testosterone. Fructose is normally produced in the seminal vesicles and secreted through the ejaculatory ducts. Spermatogenesis can be impaired by the following, resulting in an inadequate quantity or defective quality of sperm: Heat, disorders (endocrine, genetic, genitourinary), drugs (eg, anabolic steroids), toxins. Moreover we assessed the new spermatozoa production while we are assesing spermatogenesis in our tests we look at the round cell and lecocyte ratio. Round cell percentage other than leucocytes enough was determined as normal but we don't have a scale to discriminate normal from abnormal. We tought that further evaluations about this issue will take place in future guides.

There are limitations of our study that many times, 1 normal analysis will define male fertility status as normal or abnormal, for patients and providers, even though 2–3 analyses are recommended. We had only 1 test per patient performed to define fertility. The relationship between abstinence time and semen analysis results within this time frame is well-known. However in our study there was variability of abstinence time of patients therefore leading standardization differences.

Another limitation is that we didn't consider other parameters such as testicular size to evaluate fertility which was an effective parameter. We have a limitation that bio-functional sperm parameters could be altered in andrological and systemic diseases; so we had not opportunity to evaluate these in all patients either. The value of semen analysis parameters themselves has been questioned with other functional sperm abnormalities potentially evident that are independent from the current measured parameters [5,6]. It has been reported that caspase enzymatic activity is higher in semen samples with low motility [4]. Another study not only showed that LMMP correlated with sperm progressive, total motility, and volume, but also that it is possible to identify a threshold of 36.5% above which the probability of finding conventional sperm parameter abnormality increases. They also identified a threshold of HMMP of ≥ 46.25% [6].

In the WHO manual 2010, the percentage of motile spermatozoa and the proportion of progressively motile spermatozoa are assessed irrespective of speed. To ignore the speed of progressive motility is to neglect the very important qualitative mean of progressive motility. Because mean quality of the progressive motility is an important prognostic fertility factor, specifically when the proportion of motile spermatozoa is below 40% [4]. We found the mean values relating motility are shown in (**Table 4)**. In further manuals we would see the evaluation of effective circular movement and speed as in our practices.

We evaluated patients with teratozoospermia and we report that did not have a negative impact on outcomes and also did not correlate sperm concentration and total Sperm count [7].

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