Semen quality in men from subfertile couples from the region of Łódź (Poland) according to the new reference values recommended by WHO 2010

Waldemar Różański[1], Wiesław Szymczak[2], Małgorzata Wójt[3], Sławomir Sobakiewicz[3], Marek Lipiński[1], Katarzyna Marchlewska[4], Małgorzata Gołąb-Lipińska[5], Elżbieta Oszukowska[4]

[1]2nd Urology Clinic, Medical University of Łódź, Poland
[2]Institute of Psychology, University of Łódź, Poland
[3]Infertility Treatment Clinic Salve-Medica, Łódź, Poland
[4]Department of Andrology and Reproductive Endocrinology, Medical University of Łódź, Poland
[5]Department of Gynecology, Perinatology and Obstetrics, Madurowicz Hospital, Łódź, Poland

KEY WORDS

semen quality ◗ subfertility ◗ sperm concentration ◗ sperm motility

ABSTRACT

The semen analysis is the main diagnostic tool for evaluating the male fertility potential. The standard semen analysis includes evaluation of the sperm concentration, motility, and their morphology. The most important question is whether the results from semen analysis may be accurate markers for male fertility. Therefore, we retrospectively studied sperm quality among men attending the infertility clinic due to reproductive problems consistent with the WHO manual from 1999, which were reassessed according to the manual from 2010. Semen results from 571 males from couples undergoing fertility investigation were analyzed. All subjects included in the study had no abnormalities during examination. In 64 samples (11.2%), a leukocyte count above 1 x 10^6/ml was found and their semen volume (median 3.2 ml) was significantly lower in comparison with the group without leukocytes (3.6 ml; p <0.001). Normal semen parameters were found in 290 subjects (50.8%) according to the 1999 manual and in 362 men (63.4%) according to the 2010 manual. The normozoospermia group, according to the 2010 manual, had a significantly lower percentage of sperm with progressive motility, motile sperm concentration, and total number of motile sperm in comparison with the normozoospermia group according to the manual from 1999. It seems that routine semen analysis is not sufficient to estimate male fertility potential and some men with normal semen parameters may be subfertile. Further investigations are needed.

INTRODUCTION

About 10% of marriages are childless because of subfertility or infertility and a number of couples are treated for infertility. The couples' infertility depends on the reduced fertility potential of the male or female independently or of both partners collectively. Semen analysis is the main diagnostic tool for evaluating the male fertility potential. The standard semen analysis includes evaluation of the sperm concentration, motility, and their morphology. A reduced number or motility of sperm may lead to diminished male fertility [1].

Semen quality depends on some factors that cannot be modified, like sperm production by the testes. Semen analysis results will be disrupted when an incomplete sample is collected, especially when the first portion of the ejaculate is lost because it is composed mainly of the sperm-enriching fluids of the prostate gland and has a greater impact on semen quality. The presence of granulocytes in semen suggests an ongoing inflammatory process in the reproductive tract [1]. The inflammation process within accessory sex organs and their abnormal fluid secretions may change semen results, mainly progressive motility of spermatozoa. Progressive motility is essential to the efficient passage of spermatozoa through cervical mucus [2].

The process of sperm formation in the testis is recognized as a marker of human reproductive health and semen analysis is used in epidemiological research. It is currently believed that some environmental factors such as xenoestrogens may negatively influence testis function and result in a decrease in sperm production, but also increase the risk of testicular cancer development [3, 4]. In 1993, Carlsen et al. published a report suggesting decreased sperm parameters during the last 50 years [5]. In a meta-analysis of 61 semen quality studies, a decline in mean sperm density from 113 x 10^6/ml in 1940 to 60 x 10^6/ml in 1990 was observed. Moreover, during this time period they noticed an increase in the percentage from 6% to 18% of men with sperm counts lower than 20 x 10^6/ml. Many different retrospective studies were concurrently conducted and conflicting results were obtained, some confirming and others rejecting the theory concerning sperm number decline [6-10]. Lastly, studies carried out with healthy volunteers in the Northern-Baltic area showed differences in sperm number between countries and a high frequency of suboptimal semen parameters among young Danish men [11, 12]. There are very sparse data about semen quality among Polish men that was obtained during toxicity studies [13].

However, the most important outcome of semen production is fecundity of men. The question about fertility rates, reasons for fecundity decline, and the role of semen quality is still open and inconsistent results are presented [14, 15]. The most important question is whether results from semen analysis may be a certain marker for male fertility.

The World Health Organization has published "The WHO laboratory manual for the examination of human semen and sperm cervical mucus interaction" describing procedures of seminal analysis. In 2010, WHO published the fifth revised edi-

tion of this manual [16, 17]. The last edition contains many details on laboratory methods, and many efforts have been made to standardize the procedures of semen analysis, including quality control. It also includes a new reference range of semen parameters.

Therefore, we retrospectively studied sperm quality among men attending the infertility clinic due to reproductive problems consistent with the WHO manual from 1999, which were reassessed according to the manual from 2010.

MATERIAL AND METHODS

Semen results from 571 males from couples undergoing fertility investigation in Salve-Medica were analyzed. All subjects included in the study had no abnormalities during examination. The semen samples were collected after 4-days of sexual abstinence in sterile plastic containers by masturbation in the privacy room adjacent to the laboratory.

The samples were maintained at 37°C until assay. The semen analysis was performed just after liquefaction, according to WHO guidelines (1999) [16, 17]. The ejaculate volume was estimated in calibrated tubes. pH was assessed by using pH indicator strips (Merck, Germany). For the assessment of sperm motility, 10-microliters of well-mixed semen was placed on a glass slide with a coverslip. The preparation was immediately examined under 400x magnification with a microscope. The sperm were classified as motile type a when showing rapid forward motility (above 25 um/s) or type b when showing slow or sluggish forward motility (WHO 1999 motility classes). The percentage of each type in a count of 200-sperms was assessed in duplicate and the average value was calculated. According to the 2010 manual, sperm motility was classified as progressive (mean a+b) or non-progressive (Table 1). Using the Makler counting chamber (Sefi Medical Instruments, Haifa, Israel), the sperm concentration was estimated in duplicate and the average was calculated. On stained slides, the number of leukocytes was evaluated per number of sperm and thereafter calculated to represent a value in 10^6/ml. All samples were estimated by the same person (MW).

The following semen parameters were evaluated in the present study: the pH, volume of ejaculate, sperm concentration (x10^6/ml), total sperm number (sperm density x volume), percentage of progressive motility (type a+b), motile sperm concentration (sperm density x percent of progressive motility), and total motile sperm count (motile sperm concentration x volume). Semen samples that fulfilled the criteria from table 1 were included into the normozoospermia group.

Statistica (StatSoft, Kraków, Poland) was used for statistical analysis. Distribution of data was estimated by the Shapiro-Wilk test. Non-normal distribution of data was found and the results were presented as a median value (5-95 percentile) and as a mean (±SD), to facilitate comparison with studies of other authors. Differences were tested by a non-parametric test (U Mann-Whitney).

RESULTS

Men ranged from 18 to 43 years old. In 64 samples (11.2%), a leukocyte count above 1x10^6/ml was found (range 1.5 – 6 x 10^6/ml). Descriptive data of semen parameters in men with high leukocyte count and 507 samples (88.8%) with normal leukocytes count are presented in table 2. Men with high leukocyte count in semen had significantly lowered semen volume in comparison to the group without leukocytes (table 2) and they were excluded from further analysis.

Table 1. Lower reference limit for semen characteristics according to WHO manuals from 1999 and 2010.

Parameter	WHO manual 1999	WHO manual 2010
Semen volume (ml)	2.0	1.5
Total sperm count (x10^6/ejaculate)	40	39
Sperm concentration (x10^6/ml)	20	15
Motility (%)	a – 25% or a+b – 50%*	PR 32%*
pH	≥7.2	≥7.2

*a+b is the same as PR (progressive motility)

Table 2. Semen parameters in men with leukocyte count in semen higher than 1 x 10^6/ml and men with leukocyte count below or equal 1 x 10^6/ml.

Parameter		Leukocytes>1 x 10^6/mln = 64 (11.2%)	Leukocytes ≤1 x10^6/mln = 507 (88.8%)
pH	Mean ±SD	7.4 ±0.5	7.5 ±0.3
	Median	7.4	7.4
	5-95 percentile	7.0-7.9	7.2-7.9
Semen volume (ml)	Mean ±SD	3.2 ±1.6	3.6 ±1.7
	Median	2.5[a]	3.0
	5-95 percentile	1.0-6.0	1.5-7.0
Sperm concentration (x 10^6/ml)	Mean ±SD	37.2 ±25.9	37.9 ±32.9
	Median	31.0	29.0
	5-95 percentile	6.5-81	5.0-100
Total sperm number (x 10^6/ejaculate)	Mean ±SD	114.6 ±86.5	130.7 ±117.7
	Median	106.0	94.5
	5-95 percentile	15-266	10-367
Percentage of forward motility (a+b) (%)	Mean ±SD	48.8 ±13.7	49.6 ±16.1
	Median	52.0	55.0
	5-95 percentile	20-65	10-65
Motile sperm concentration (x 10^6/ml)	Mean ±SD	19.6 ±15.4	20.5 ±19.6
	Median	14.7	15.2
	5-95 percentile	1.5-49.2	1.0-57.0
Total motile sperm count (x 10^6/ejaculate)	Mean ±SD	60.8 ±52.2	70.6 ±69.9
	Median	49.3	48.6
	5-95 percentile	3.2-146.3	2.9-213.8

a – p <0.001 (U Mann-Whitney test)

Normal semen characteristic (normozoospermia) was found in 290 subjects (50.8%) according to the 1999 manual and in 362 men (63.4%) according to 2010 manual. Their semen parameters are presented in table 3.

According to the 1999 manual, abnormal semen parameters were found in 217 (38.0%) men; 108 (18.9%) men had lowered sperm concentration (oligozoospermia) and 4 (0.8%) of them had no sperm (azoospermia); 96 (16.8%) had decreased sperm motility (asthenozoospermia) and 13 (2.3%) men had low semen volume (parvisemia). Semen parameters of men with abnormal results are presented in table 3. According to the 2010 manual, abnormal semen parameters were found in 145 (25.4%) men; oligozoospermia was diagnosed in 107 (18.7%), asthenozoospermia in 33 (5.8%), and parvisemia in 5 (0.9%). The parameters of their semen are presented in table 3.

Table 3. Semen parameters in men with normozoospermia and abnormal semen parameters according to the WHO manual from 1999 and 2010

Parameter		Normozoospermia		Abnormal semen parameters	
		WHO 1999 n = 290 (50.8%)	WHO 2010 n = 362 (63.4%)	WHO 1999 n = 217 (38.0%)	WHO 2010 n = 145 (25.4%)
pH	Mean ±SD	7.5 ±0.2	7.5 ±0.2	7.4 ±0.3	7.4 ±0.3
	Median	7.4	7.4	7.4	7.4
	5-95 percentile	7.2-7.9	7.0-7.8	7.0-7.9	7.1-8.0
Semen volume (ml)	Mean ±SD	3.9 ±1.6	3.9 ±1.7	3.2 ±1.7	2.9 ±1.4
	Median	3.5	3.5	3.0	2.5
	5-95 percentile	2.0-7.0	2.0-7.0	1.0-7.0	2.0-5.5
Sperm concentration (x 10^6/ml)	Mean ±SD	48.4 ±34.0	46.3 ±33.5	23.8 ±25.4	16.8 ±19.1
	Median	38.5	37.0	15.5	9.5[b]
	5-95 percentile	16-108	14-106	1.5-85.0	0.5-58.0
Total sperm number (x 10^6/ejaculate)	Mean ±SD	174.3 ±120.8	165.6 ±118.1	72.3 ±83.5	43.4 ±54.4
	Median	140.0	130 .0	40.0	28.0[b]
	5-95 percentile	48-400	45.5-390	2.5-236.5	0,75-164.5
Progressive motility (a+b) (%)	Mean ±SD	58.7 ±6.1	55.6 ±8.9	37.4 ±17.3	34.7 ±19.9
	Median	60.0	55.0[a]	40	35
	5-95 percentile	50-70	40-70	0.0-60	0.0-60
Motile sperm concentration (x 10^6/ml)	Mean ±SD	28.6 ±20.3	26.2 ±19.8	9.6 ±11.9	6.0 ±8.8
	Median	22.0	20.4[a]	5.3	3.2[b]
	5-95 percentile	8.8-69.0	6.3-65.0	0.0-34.2	0.0-24.6
Total motile sperm count (x 10^6/ejaculate)	Mean ±SD	103.0 ±72.7	93.5 ±70.2	27.3 ±33.4	13.6 ±15.9
	Median	79.9	72.0[a]	16.5	9.8[b]
	5-95 percentile	28.8-240.0	24.0-231.0	0.0-94.5	0.0-49.4

a – $p < 0.001$ *vs.* normozoospermia according to WHO 1999
b – $p < 0.001$ *vs.* abnormal semen parameters according to WHO 1999 (U Mann-Whitney test)

Percentage of sperm with progressive motility, motile sperm concentration, and total number of motile sperm were significantly lower in the normozoospermia group according to the 2010 manual in comparison with the normozoospermia group according to the 1999 manual.

DISCUSSION

Among 571 men from couples with decreased reproductive capacity normozoospermia was diagnosed in 50.8% or 63.4% (according the 1999 or 2010 manual, respectively) with median sperm concentration 38.5 x10^6/ml or 37.0 x10^6/ml (table 3), respectively. These numbers are lower than described previously among young men from: Finland – 54 x 10^6/ml; Estonia – 57 x 10^6/ml; Denmark – 41 x 10^6/ml; or Norway – 41 x 10^6/ml [11, 12]. Moreover, median sperm concentration in studied men was lower than in partners of pregnant women from the Warsaw region (64 x 10^6/ml) [13]. This may be a result of a selection bias (men from couples without reproductive problems) or it may be connected with an influence from other factors.

An important function of semen result is prognosis, i.e. whether a man and his wife are going to be able to conceive naturally and how long it is likely to take. Many studies evaluating time to pregnancy (TTP) were done among couples that successfully conceived. In the prospective study of first-pregnancy planners, Bonde et al. showed shortening of TTP in subjects with higher sperm density up to the level of 40 x 10^6/ml [18]. On the contrary, in Norwegian fertile males (partners of pregnant women within TTP <12) the 5th percentile for sperm concentration was 10.6 x 10^6/ml and progressive motility was 33.2% [19]. These values are lower than in our normozoospermia groups. They also showed that men from couples with TTP = 1 had a significantly higher total number of motile sperm (median 238 x 10^6/ejaculate) than other fertile men, and this value is 2-fold higher than in our studied groups. Similarly, Bartoov et al. showed significantly higher total sperm number (201 x 10^6/ejaculate) and percentage of forward motility (46.6%) in men, who experienced pregnancy in a time shorter than 12-months in comparison with men who did not obtain pregnancy during a time longer than 5-years [20]. They concluded that assessment of several parameters simultaneously allows for a more precise evaluation of men's fertility potential. The values of semen parameters in the group of fertile men presented by Bartoov et al. did not considerably differ from values observed in our normozoospermia group. Other authors have shown that thresholds for sperm concentration depend on statistical methods and vary from 9 to 34 x 10^6/ml, and for motility from 20 to 52% [21, 22]. It was shown that TTP may depend on some other factors. TTP was significantly longer among citizens of Paris than among inhabitants of Copenhagen, Turku, or Edinburgh, but no differences in semen parameters were found [23]. Similar results have been obtained during the study on environmental pollutions [13]. TTP changes did not relate to semen parameters or body levels of pollutions. These observations suggest that TTP does not seem to be an adequate parameter to assess male or female fertility. Currently epidemiologists stress the bio-social determi-

nants of fertility, namely, socio-economic trends that postpone childbearing, fertility-related behavior, persistent stress, and some genetic influences [15, 24].

Semen progressive motility is crucial for the efficient passage of spermatozoa through cervical mucus [2]. In the 1999 edition of the WHO manual, spermatozoa with fast and slow progressive movement were distinguished from sperms with non-progressive movement or non-motile. According to the 2010 manual only three classes of sperm movement are distinguished: progressive, non-progressive, and immotile. This division is easier for learning and performing [1]. Additionally, the limit of percentage of sperm with progressive motility (a+b according to the manual from 1999) was lowered.

According to the latest WHO manual, more than half the studied males were diagnosed with normal semen parameters. Does the diagnosis of normozoospermia means that the fertility potential of that male is normal? This would mean that man's sperm is able to make his partner pregnant. It was shown that men's likelihood of fathering a child might depend on some additional factors like DNA fragmentation index (DFI). Giwercman et al. showed that in normozoospermic men, a DFI higher than 20% resulted in decreased fertility with an odds ratio of five and that DFI may be an independent predictor of natural conception [25]. Other tests describing the functional competence of sperm that are necessary for conceiving have been described by Aitken [26].

Sperm density below 20 x 10^6/ml was found in 18.7% of the studied men and this value was slightly lower than that observed among Danish candidates for military service (21%) according to Andersen et al. [12]. Men in our group came from infertile/subfertile couples, but this did not result in a selection of subjects with a very low sperm count. This finding supports the thesis that male fecundity is not only related to sperm count.

Francavilla et al. analyzed inter-subject variability of semen parameters in men from infertile couples who had experienced successful intrauterine insemination [27]. They showed that total motile sperm count was negatively affected on the second day of abstinence. Additionally, they noticed that one day of sexual abstinence resulted in the decrease of semen volume, but improved sperm quality in most cases of oligoasthenozoospermia. It supports the thesis that prolonged storage of sperm in the epididymis may lead to sperm damage and was previously described by Johnson and Varner [28]. They showed that in men with a low sperm production rate within testis, the time of sperm transport through the epididymis becomes longer and may lead to excessive "aging" of sperm. This may result in poorer semen parameters in men with oligozoospermia.

In 64 males, an abnormally high number of leukocytes were found, suggesting an ongoing inflammatory process in the reproductive tract. A significantly smaller volume of semen was observed in these men when compared to men without leukocytes and is related to abnormal function of male accessory sex glands [1]. Notably, the diagnosis was established in these men and effective treatment will be initiated.

It seems that routine semen analysis is not sufficient in estimating male fertility potential and some men with normal semen parameters may by subfertile. Moreover, the diagnostic value of the semen result is limited due to its lack of information about the cause of abnormalities and the mechanism of injury to sperm formation or function.

Supported by Medical University of Łódź funds No 503-0157-1, 502-11-427, 503-1089-2/3

REFERENCES

1. Björndahl L: *The usefulness and significance of assessing rapidly progressive spermatozoa*. Asian J Androl 2010; 12: 33-35.
2. Keel BA, Webster BW: *Correlation of human sperm motility characteristics with an in vitro cervical mucus penetration test*. Fertil Steril 1988; 49: 138-143.
3. Słowikowska-Hilczer J: *Xenoestrogens and male reproduction*. ANIR 2006; 8 (1): 32-39.
4. Skakkebaek NE, Rajpert-De Metys E, Jorgensen N et al: *Germ cell cancer and disorders of spermatogenesis: an environmental connection?* APMIS 1998; 106: 3-12
5. Carlsen E, Giwercman A, Keiding N, Skakkebaek N: *Evidence for decreasing quality of semen during past 50 years*. Br Med J 1992; 305: 609-613.
6. Adamopoulos DA, Pappa A, Nicopoulou S et al: *Seminal volume and total sperm number trends in men attending subfertility clinics in the greater Athens area during the period 1977-1993*. Hum Reprod 1996; 11: 1936-1941.
7. Van Waeleghem K, De Clercq N, Vermeulen L et al: *Deterioration of sperm quality in young healthy Belgian men*. Hum Reprod 1996; 11: 325-329.
8. Gandini L, Lombardo F, Culasso F et al: *Myth and reality of the decline in semen quality: an example of the relativity of data interpretation*. J Endrocrinol Invest 2000; 23: 402-411.
9. Paulsen CA, Berman NG, Wang C: *Data from men in greater Seattle area reveals no downward trend in semen quality: further evidence that deterioration of semen quality is not geographically uniform*. Fertil Steril 1996; 65: 1015-1020.
10. Rasmussen PE, Erb K, Westergaard LG, Laursen SB: *No evidence for decreasing semen quality in four birth cohorts of 1055 Danish men born between 1950 and 1970*. Fertil Steril 1997; 68: 1059-1064.
11. Jorgensen N, Carlsen E, Nermoen I et al: *East-West gradient in semen quality in the Nordic-Baltic area: a study of men from general population in Denmark, Norway, Estonia and Finland*. Hum Reprod 2002; 17: 2199-2208.
12. Andersen AG, Jensen TK, Carlsen E et al: *High frequency of sub-optimal semen quality in an unselected population of young men*. Hum Reprod 2000; 15: 366-372.
13. Toft G, Axmon A, Giwercman A et al: *Fertility in four regions spanning large contrasts in serum levels of widespread persistent organochlorines: a cross sectional study*. Environm Health 2005; 4: 26-38.
14. Skakkebaek NE, Jørgensen N, Main KM et al: *Is human fecundity declining?* Int J Androl 2006; 29: 2-11.
15. *General discussion: Role of poor semen quality for current infertility and future fertility rates – lessons from the clinic and current population studies*. Int J Androl 2006; 29: 105-108.
16. *WHO Laboratory manual for the examination of human semen and sperm-cervical mucus interaction*. World Heath Organization. Cambridge University Press, Cambridge, 1999: 4-26.
17. *WHO laboratory manual for the examination and processing of human semen*. Fifth Edition. World Health Organisation, 2010: 1-114
18. *Bonde JP, Ernst E, Jensen TK et al: The relation between semen quality and fertility. A population based study of 430 first-pregnancy planners*. Lancet 1998; 352: 1172-1177.
19. Haugen TB, Egeland T, Magnus O: *Semen parameters in Norwegian fertile men*. J Androl 2006; 27: 66-71.
20. Bartoov B, Eltes F, Pansky M et al: *Estimating fertility potential via semen analysis data*. Hum Reprod 1993, 8 (1): 65-70.
21. Ombelt W, Bosmans E, Janssen M et al: *Semen parameters in a fertile versus subfertile population: need for change in the interpretation of semen testing*. Hum Reprod 1997; 12: 987-993.
22. Guzick DS, Overstreet JW, Factor-Litvak P et al: *Sperm morphology, motility and concentration in fertile and infertile men*. N Eng J Med 2001; 345: 1388-1393.
23. Jensen TK, Slama R, Ducot B et al: *Regional differences in waiting time to pregnancy among fertile couples from four European cities*. Hum Reprod 2001; 16 (12): 2697-2704.

24. Kohler HP, Rodgers JL, Miller WB et al: *Bio-social determinants of fertility*. Int J Androl 2006: 29: 46-53.
25. Giwercman A, Lindstedt L, Larsson M et al: *Sperm chromatin structure assay as an independent predictor of fertility in vivo: a case control study*. Int J Androl 2010; 33: e221-e227.
26. Aitken RJ: Sperm function tests and fertility. Int J Androl 2006; 29: 69-75.
27. Francavilla F, Barbonetti A, Necozione S et al: *Within-subject variation of seminal parameters in men with infertile marriages*. Int J Androl 2007; 30: 174-181.
28. Johnson L, Varner DD: *Effect of daily spermatozoan production but not age on transit time of spermatozoa through the human epididymis*. Biol Reprod 1988; 39: 812-817.

Correspondence

Elżbieta Oszukowska
Department of Andrology and Reproductive Endocrinology
5, Sterling Street
92-425 Łódź, Poland
phone: +48 42 633 07 05
elzbieta.oszukowska@umed.lodz.pl