BASIC SCIENCE

EVALUATION OF SEMEN QUALITY IN YOUNG MEN IN REPUBLIC OF NORTH MACEDONIA

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Abstract

Research data show that in the last 50 years (1938-1991) there has been a trend of decreasing sperm concentration in the male population in Europe by 2.3% and in the USA by 0.8%. The reasons for such negative trend are not known, but it is assumed that lifestyle and environmental factors have an influence on genetic factors. Aim of this study was to evaluate sperm quality in young, healthy men in our country, and to compare sperm quality in our population with others in the world. Material and methods: Ejaculates from 203 healthy male subjects, aged 18-32, were stored in a thermostat at 36^{0}C and analyzed manually on a native slide and hematoxylin-eosin-stained slides, under a phase contrast microscope. Sperm motility was assessed at two-time intervals, group A, 60 minutes after ejaculation and group B, 120 minutes after ejaculation, while sperm concentration and sperm morphology were assessed at one time interval. Results:Semen analysis showed an average volume of ejaculate3.45 ± 1.5 ml, sperm concentration in 1 milliliter62.4 ± 39.2 x10(6) /ml, while total sperm concentration was 211.2 ± 173.2 x10(6). In group A, values for progressive spermatozoa were 48.6 ± 18.1 x10(6) /ml and in group B, values for progressive spermatozoa were 47.9 ± 17.3 x10(6) /ml. There was no statistically significant difference between the two time intervals (group A and group B) when interpreting sperm motility, p>0.005. Analysis of morphology of spermatozoa showed a mean value of 6.9% for morphologically normal spermatozoa. Conclusion: The quality of ejaculate in young men in North Macedonia is in the range of reference values according to WHO, and also our results are similar to those from Germany, Turkey, Bulgaria, Faroe Islands.

БАЗИЧНИ ИСТРАЖУВАЊА

ЕВАЛУАЦИЈА НА КВАЛИТЕТОТ НА ЕЈАКУЛАТ КАЈ МЛАДИТЕ МАЖИ ВО РЕПУБЛИКА СЕВЕРНА МАКЕДОНИЈА

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Клучни зборови: подвижност на сперматозоидите, концентрација на сперматозоидите, морфологија на сперматозоидите.

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Извадок

Истражувачките податоци покажуваат дека во последните 50 години (1938-1991) постои тренд на намалување на концентрацијата на сперматозоиди кај машката популација во Европа за 2,3% и во САД за 0,8%. Причините за ваквиот негативен тренд не се познати, но се претпоставува дека начинот на живот и факторите на околината имаат влијание врз генетските фактори. Целта на оваа студија беше да се оцени квалитетот на ејакулатот кај млади, здрави мажи во нашата земја, за да можеме да го споредиме квалитетот на ејакулатот кај нашата популација со другите популации во светот. Материјал и методи: Ејакулите од 203 здрави машки испитаници, на возраст од 18-32 години, беа складирани во термостат на 36^{0}C и рачно анализирани на нативен препарати препарати обоени со хематоксилин/еозин, под фазно-контрастен микроскоп. Подвижноста на сперматозоидите беше проценета во два временски интервала, група А, 60 минути по ејакулацијата и група Б, 120 минути по ејакулацијата, додека концентрацијата и морфологијата на сперматозоиди беа анализирани во еден временски интервал. Резултати: Анализата на ејакулатите покажа просечен волумен на ејакулатот 3,45 ± 1,5 ml, концентрација на сперматозоиди во 1 милитар 62,4 ± 39,2 x10 (6) / ml, додека вкупната концентрација на сперматозоиди беше 211,2 ± 173,2 x10 (6). Во групата А, вредностите за прогресивни сперматозоиди беа 48,6 ± 18,1 x10 (6) /ml, во групата Б, вредностите за прогресивни сперматозоиди беа 47,9 ± 17,3 x10 (6) /ml. Немаше статистички значајна разлика помеѓу двата временски интервала (група А и група Б) при интерпретација на подвижноста на сперматозоидите, p>0,005. Анализата на морфологијата на сперматозоидите покажа вредност од 6,9% за присуство на морфолошки нормални сперматозоиди. Заклучок: Квалитетот на ејакулатот кај младите мажи во Северна Македонија е во опсегот на референтните вредности според СЗО. Нашите резултати се слични на оние од Германија, Турција, Бугарија, Фарските Острови.

Introduction

World literature points to the fact that in many developed countries there is a trend of declining fertility among the male population[1]. The quality of the ejaculate reflects the fertile ability of the male individual in the fertilization process. Numerous factors influence the achievement of this task. The amount of seminal fluid, the number of spermatozoa, their quality in terms of shape, vitality, motility are some of the characteristics that reflect the ability to fertilize[2]. However, many other factors, influence this complex process.

Research data show that in the last 50 years (1938-1991) there has been a trend of decreasing sperm concentration in the male population in Europe by 2.3% and in the USA by 0.8%[3]. It is considered that between 19 - 29 years the concentration of spermatozoa is constant, but after that, decreased values have been observed mainly in sperm concentration[4,5].

Laboratories and centers for *in vitro* fertilization which study this issue, use data given by the WHO as reference values[6,7]. Those values are obtained from studies of people from different parts of the world in which there are different climatic conditions, different way of life, working conditions, diet, religious customs, traditions and so on. Studies have shown that there is a difference in the same age groups of respondents from different countries[8,9,10]. The reasons for such regional differences are not known, but it is assumed that regional, lifestyle and environmental factors have an influence on genetic factors[11,12]. Low spermatogenic capacity may be associated with developmental disorders of the male reproductive system such as cryptorchidism, hypospadias, and testicular germ cell carcinoma, which are components of testicular dysgenesis syndrome (TDS). There is an increasing trend of TDS over the past decades in western countries. The explanation is sought in the exposure of the testicles of fetuses to environmental factors, mostly chemical ones, which lead to endocrine disorders[13].

Lifestyle factors also affect spermatogenesisand sperm quality. These factors include smoking, drinking, drug abuse, diet, obesity, chemicals, pesticides, use of cell phones and laptops. Each of these factors has negative correlation with the quality of the sperm and fertile potential in men if the person is overexposed[14].

The aim of this study was to evaluate sperm quality in young, healthy men in our country, and to compare sperm quality in our population with others in the world.

Material and methods

Participants

The study was conducted in the Laboratory for Analysis of Human Ejaculate at the Institute of Histology and Embryology, at the Faculty of Medicine in Skopje, in the period 2018-2020. Human ejaculates from 203 healthy male subjects were analyzed. Respondents included in this study were young men and students from the Faculty of Medicine (I-VI

year), Faculty of Dentistry (I-V year) and University School for physiotherapists, medical technicians, x-ray technicians (I-III year), all at the Ss. Cyril and Methodius University in Skopje.

All respondents were voluntarily included in this study and were properly informed about the entire procedure.

The study was conducted according to the following protocol, which foresees the following stages and procedures:

- The respondent signs an informed consent that he consciously and voluntarily approaches to this research
- The respondent fills out a questionnaire that contains useful data about the subject of thestudy
- The respondent receives information about conditions that must be met before delivering the material for analysis.

This research was approved by the Committee for Ethical Issues at the Faculty of Medicine in Skopje.

Materials and methods

After delivering, the material for analysis was stored in a thermostat at temperature of 36^0C. Firstly, the method of observation was used, and then microscopic analyses were performed according to the WHO Laboratory manual for the examination and processing of human semen (5^{th} edition). The analyses included procedures that determined qualitative and quantitative parameters of the ejaculate:

- Macroscopic examination of the ejaculate:
  - volume
  - liquefaction
  - pH
  - viscosity

- Microscopic examination of the ejaculate:

Initial microscopic analysis was performed using a phase-contrast, light microscope, with magnification X40, X60 and X100, on native and hematoxylin-eosin stained slides. On native slides, we analyzedsperm motility and sperm concentration. On hematoxylin-eosin stained slides, we analyzed sperm morphology. Sperm vitality was assessed using the eosin-nigrosin method of staining.

- Sperm motility

  Sperm motility was determined in at least 5-10 fields of view (progressive spermatozoa, non-progressive spermatozoa and immotile spermatozoa, by counting a minimum of 200 spermatozoa, according to standard procedures (WHO, 5th edition). Sperm motility was analyzed at 2-time intervals, after 60 and 120 minutes from ejaculation (group A - 60 minutes and group B - 120 minutes).

- Sperm vitality

  The percentage of vital spermatozoa in the ejaculate was determined using eosin-nigrosin method of staining, according to standard procedures (WHO, 5th edition).

- Sperm concentration

  Number of spermatozoa in 1 ml and in the entire volume of the ejaculate was determined by counting spermatozoa in an Improved Neubauer chamber according to standard procedures (WHO, 5th edition).

- Sperm morphology

  Differential morphological analysis was performed on permanent histological slides, stained with hematoxillin-eosin, by which the morphological appearance of spermatozoa was qualitatively and quantitatively determined, with a percentage representation of normal and deviant spermatozoa. On the deviant spermatozoa, we quantitatively determined the percentage of deviations of the head, midpiece, tail of the spermatozoa and presence of cytoplasmic residue (WHO, 5th edition, Kruger's strict criteria).

The obtained macroscopic and microscopic results were processed using a descriptive statistical method and t-test to present the difference between the groups. Statistical analysis was performed using SPSS 17.0 software; $p<0.05$ was considered as significant.

Results

This study analyzed human ejaculates from 203 respondents, aged 18 to 32 years, with the average age of 24.3 ± 4.2 years.

Macroscopic examination of the ejaculate showed that the average ejaculate volume was 3.45 ± 1.5 ml; the smallest measured volume was 1 ml, the maximum measured volume was 9.2 ml. 159 (78.3%) ejaculates had a normal volume, 23 (11.3%) respondents had an ejaculate with a decreased volume.

pH values ranged from 6.7 to 8, the average pH value was 7.5 ± 0.2; 188 (92.65) ejaculates had a normal pH value, 12 (5.9%) ejaculates had a pH value lower than 7.2; 3 (1.5%) ejaculates had a pH value higher than [7.8].

Liquefaction time ranged from 15 to 240 minutes, an average of 34.5 ± 17.7 minutes. In 151 (74.4%) respondents, the liquefaction time was shorter than 30 minutes, while in 50 (24.6%) respondents the liquefaction of the ejaculate was longer than 30 minutes.

Viscosity of the ejaculate was normal in 185 (91.1%) respondents, while in 18 (8.9%) respondents it was increased.

Table 1. Macroscopic characteristics of the ejaculate

Parameter	Values	WHO 5 – reference values
Age (mean ± SD) (min – max)	(24.3 ± 4.2) (18 – 33)	
Volume / ml (mean ± SD) (min – max)	(3.45 ± 1.5) (1 – 9.2)	≥1.5
volume (%) normal (2 – 5 ml) decreased increased	 159 (78.32) 23 (11.33) 21 (10.35)	
pH (mean ± SD) (min – max)	(7.5 ± 0.2) (6.7 – 8)	7.2
pHn (%) normal (7.2 – 7.8) decreased increased	 188 (92.61) 12 (5.91) 3 (1.48)	
Liquefaction /minutes (mean ± SD)	(34.5 ± 17.7) (15-240)	30 minutes
Liquefaction n (%) <30minutes ≥30minutes	 151 (74.38) 50 (24.63)	
Viscosity		
Viscosity n (%) normal increased	 185 (91,13) 18 (8,87)	

The average sperm concentration was 62.4 ± 39.2x10(6)/ml; the lowest value of sperm concentration in 1 ml of ejaculate was 1 million spermatozoa, while the highest value of sperm concentration was 150x10(6)/ml. Lower sperm concentration than 15x10(6)/ml was detected in 36 (17.7%) of the ejaculates.

The total number of spermatozoa in the ejaculate ranged from 1 to 1196x10(6); the average value of the total number of sperm in the ejaculate was 211.2 ± 173.2 x10(6). Total sperm count in the ejaculate was decreased (<39x10(6)) in 30 (14.8%) respondents.

The number of progressive spermatozoa in 1ml ranged from 0.01 to 693x10(6)/ml; average 38.4 ± 52.8x10(6)/ml. Decreased number of progressive spermatozoa (<10x10(6)/ml) was detected in 37 (18.2%) ejaculates.

The total number of progressive spermatozoa in the ejaculate averaged 119.1 ± 106.5x10(6); the lowest total number of progressive spermatozoa was 0.038x10(6), while the highest was 660x10(6). In 36 (17.7%) ejaculates, the total number of progressive spermatozoa was decreased, it was less than 30x10(6) (Table 2).

Table 2. Sperm concentration and sperm motility

Parameter	n (%)	WHO 5 – reference values
Sperm concentration/x10(6) /ml (mean ± SD) (min – max)	(62.4 ± 39.2) (1-150)	≥ 15 x10(6)/ml
Sperm concentration (/ml)n (%) <15 >15	36 (17.73) 167 (82.27)	
Total sperm concentration/ x10(6) (mean ± SD) (min – max)	(211.2 ± 173.2) (1 – 1196)	≥39 x10(6)
Total sperm concentration/ x10(6)n (%) <39 >39	30 (14.78) 173 (85.22)	
Progressive spermatozoa/ x10(6)/ml (mean ± SD) (min – max)	(38.4 ± 52.8) (0.01 – 693)	≥ 32%
Progressive spermatozoan (%) <15 >15	37 (18.23) 164 (80.79)	
Total progressive spermatozoa/ x10(6) (mean ±SD) (min – max)	(119.1 ± 106.5) (0.038 – 660)	
Total progressive spermatozoan (%) <30 >30	36 (17.74) 165 (81.28)	

In the analysis performed in the first 60 minutes after ejaculation, the number of progressive spermatozoa ranged from 3 to 88%, average 48.6 ± 18.1%; the number of non-progressive spermatozoa ranged from 1 to 23%, average 6.5 ± 3.4%; the number of viable spermatozoa ranged from 9 to 97%, average 43.1 ± 17.2%. In these analyses, in 91 (44.8%) ejaculates a reduced percentage of progressive spermatozoa was observed (<50%), in 17 (8.4%) ejaculates an increased percentage of non-progressive spermatozoa was observed (>10%), while in 115 (56.65%) ejaculates an increased percentage of possessive spermatozoa (> 40%) was observed.

In the analysis performed in the second hour (120 minutes) after ejaculation, the number of progressive spermatozoa ranged from 2 to 87%, average 47.9 ± 17.3%; the number of non-progressive spermatozoa ranged from 1 to 30%, average 6.8 ± 3.2%; the number of possessive spermatozoa ranged from 11 to 100%, average 44.3 ± 17.4%. In these analyses, in 100 (49.3%) ejaculates, a reduced percentage of progressive spermatozoa (<50%) was observed, in 13 (6.4%)

ejaculates an increased percentage of non-progressive spermatozoa (>10%) was observed, while in 115 (56.65%) ejaculates an increased percentage of possessive spermatozoa (> 40%) was observed (Table 4). In 14(6.7%) ejaculates spermatozoa had reduced vitality (<45%) (Table 4).

When progressive spermatozoa were compared at 60 and 120 minutes (group A and group B), there was no statistically significant difference between the two groups, t=0,4028; p=0.6873;p>0.005 (Table 3).

Table 3. Comparison of progressive sperm motility at 60 and 120 minutes

	$(\bar{x})$	(Σ)	St.error	95% confidence interval of difference		t	df	p
Group 1 (60 min) – group 2 (120min)	48.250	17.500	1.738	-2.716	4.116	0.4028	404	0.6873

Table 4. Sperm motility and sperm vitality

	n (%)	WHO 5 - reference values
Progressive spermatozoa (60minutes) (mean ± SD) (min – max)	(48.6 ± 18.1) (3 – 88)	≥ 32%
Progressive spermatozoa (60 minutes)n (%) ≥ 32% <32%	 109 (53.69) 91 (44.83)	
Non-progressive spermatozoa (60 minutes) (mean ± SD) (min – max)	(6.5 ± 3.4) (1 – 23)	≤ 8%
Non-progressive spermatozoa (60 minutes) n (%) ≤ 8% >8%	 184 (90.64) 17 (8.37)	
Immotile spermatozoa (60 minutes) (mean ± SD) (min – max)	(43.1 ± 17.2) (9 – 97)	
Immotile spermatozoa (60 minutes) n (%) ≤ 60% >60%	 86 (42.36) 115 (56.65)	
Progressive spermatozoa (120 minutes) (mean ± SD) (min – max)	(47.9 ± 17.3) (2 – 87)	≥ 32%
Progressive spermatozoa (120 minutes) n (%) ≥ 32% <32%	 102 (50.25) 100 (49.26)	
Non-progressive spermatozoa (120 minutes) (mean ± SD) (min – max)	(6.8 ± 3.2) (1 – 30)	≤ 8%
Non-progressive spermatozoa (120 minutes) n (%) ≤ 8% >8%	 188 (92.61) 13 (6.4)	

Immotile spermatozoa(120 minutes) (mean ± SD) (min – max)	(44.3 ± 17.4) (11 – 100)	
Immotile spermatozoa(120 minutes) n (%) ≤ 60% >60%	86 (42.36) 115 (56.65)	
Sperm vitality (mean ± SD) (min – max)	(65.6 ± 14.9) (0 – 100)	
Sperm vitalityn (%) ≤58% >58%	14 (6.89) 188 (92.61)	

Table 5. Basic differential morphological analysis of spermatozoa

Basic differential morphological forms of spermatozoa	
	n (%)
Normal spermatozoa n (%) (WHO-5 reference values) ≥4 <96	130 (64.04) 73 (35.96)
Deviant spermatozoa n (%) <96 ≥4	134 (65.51) 69 (33.99)
Head deviations n (%) <30 ≥30	184 (90.64) 19 (9.36)
Midpiece deviations n (%) <30 ≥30	169 (83.26) 34 (16.74)
Tail deviations n (%) <30 ≥30	174 (85.71) 29 (14.29)
Cytoplasmic residue n (%) <30 ≥30	189 (93.10) 14 (6.90)
Combined deviationsn (%) <40 ≥40	168 (82.76) 35 (17.24%)

Morphological analysis showed presence of 6.9% of morphologically normal spermatozoa in the ejaculates. The results of the differential morphological analysis of spermatozoa found 130 (64%) ejaculates with normal shape of spermatozoa. In the remaining 73 (35.96%) ejaculates, the presence of deviations in the heads, midpiece and tails of the spermatozoa was observed, as well as cytoplasmatic residue. Analysis of the sperm

head showed presence of this deviation in 19 (9.36%) ejaculates, analysis of the midpiece showed presence of this deviation in 34 (16.74%)ejaculates, while analysis of the tails of the spermatozoa showed deviation in 29 (14.29%) ejaculates. Presence of cytoplasmic residue in spermatozoa was noticed in14 (6.90%) ejaculates.Combined sperm abnormalities were observed in 35 (17.24%) ejaculates (Table 5).

Table 6. Semen quality in young men in North Macedonia

Total number of respondents N (203)	Age (years)	Volume (ml)	Sperm concentration (million/ml)	Total sperm concentration (million)	Progressive spermatozoa (million/ml)	Morphology (%)
Mean (SD)	24.3 (4.2)	3.45 (1.5)	62.4 (39.2)	211.2 (173.2)	38.4 (52.8)	6.9 (3.6)
Median	24	3	62	207	37.1	7.2

Discussion

Evaluation of the parameters of the spermogram is one of the best indicators of male reproductive health15. A large number of studies from different countries worldwide point to a declining trend in semen quality; therefore,an early screening of young men for semen quality can contribute to preserving and improving fertility[3].

We conducted this study in order to gain insight into the fertilecapacity of young men in North Macedonia and to compare the obtained results with the latest reference values of the World Health Organization, as well as with other studies on this topic in the world.

Our study comprised young healthy men aged 18-32, because many studies suggest that spermatogenesis in men at this age is at its highest level [4,5]. Volume of the semen fluid was within normal reference values, according to WHO guidelines,3.45 ± 1.5ml (mean ±SD), as it was in the study of Mendiolaet *al.*, Jorgensen et al. and Rao et *al* [1,8,11].

The values for sperm concentration in 1 milliliter of ejaculate were 62.4 ± 39.2 x10(6) /ml (mean ± SD), which is in accordance with the WHO reference values, ≥15 x10(6) /ml. The total sperm concentration in the ejaculate was 211.2 ± 173.2 x10(6) (mean ± SD), >39 x10(6). Similar values for sperm concentration per milliliter of ejaculate and total sperm concentration were reported in the studies by Jiang et al. and Hallinget *al.*[16,21].

Most studies report assessing sperm motility over a single time interval. In our study, we assessed sperm motility at two-time intervals. The first time interval, group A - 60 minutes after ejaculation, showed 48.6 ± 18.1% for progressive spermatozoa and 6.5 ± 3.4% for non-progressive spermatozoa. At the second time interval, group B - 120 minutes after ejaculation, results for progressive spermatozoa were 47.9 ± 17.3% and for non-progressive spermatozoa 6.8 ± 3.2%. There was no statistically significant difference between the two

time intervals (group A and group B) when interpreting sperm motility, t=0.4028; p=0.6873; p>0.005. The obtained values for sperm motility were within the WHO reference values. Similar values for sperm motility were shown in the study by Li et *al.*[7]. Sperm viability tests showed values within the WHO reference values, ≥58. The values were 65.6 ± 14.9%. Sperm morphology analysis detected presence of 6.9% of morphologically normal spermatozoa, which is within the WHO reference values, according to Kruger's strict criteria. In 130 ejaculates, normal morphological forms of spermatozoa were found, with the presence of >4% of morphologically normal forms of spermatozoa, while in 73 ejaculates deviant morphological forms of spermatozoa were found, ≥96%. Guzick et *al*. in their study noticed presence of morphologically normal forms of spermatozoa higher than 12% in fertile men[18]. Assessing morphologicallydeviant forms, we found that sperm head deviations, >30%, were noted in 19 ejaculates, sperm midpiece deviations, >30%, were noted in 34 ejaculates, sperm flagellum deviations, >30%, in 29 ejaculates, while presence of sperm cytoplasmic residue, >30%, was noted in 14 ejaculates. The presence of combined deviations, >40%, was found in 35 ejaculates. For 20 years, sperm morphology assessment has been described by some authors as a good indicator of male fertility[19]. Data from our study show higher values of sperm motility and concentration, and lower value of sperm morphology compared to the study of Dobrinov et al., conductedon young men from Bulgaria[20]. Our findings about the quality of the ejaculate in young men were similar to those presented inthe study of Halling J et *al.*[21], done with young men in the Faroe Islands. The resultsof our study for sperm volume and sperm concentration were also similar to the results in the study performed by Paasch et al.,which referred to young men in Germany[22]. A higher value for sperm concentration was shown in the study by Cok et *al.*[23], which involved a population of young men in Turkye, as well as in the study made by Li et *al.* which comprised young men in China[17].

Conclusion

The results presented in this study have given a realistic picture for the quality of the ejaculate in young, healthy men from our region. These results are characteristic for the young male population with the same or similar conditions in lifestyle, work, diet and tradition typical for the Republic of North Macedonia.

These initial results allow us to compare them with those obtained in other countries in the world, so we can conclude that the quality of ejaculate in young men in North Macedonia is within the WHO reference values, and also our results are similar to those from Germany, Turkey, Bulgaria, Faroe Islands.

These results will be supplemented with data in the next few years, in order to establish reference values for the parameters of the spermogram of the young male population in North Macedonia.

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