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Evaluation of the semen quality of patients attended in a clinical analyzes in the Cascavel municipality, Brazil

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Abstract

Objectives: The objective of this study was to investigate the prevalence of altered spermograms of individuals living in a municipality in the west of Paraná and attended at a private laboratory for clinical analysis, as well as the main alterations found in this study. **Methods:** We analyzed electronic reports of patients undergoing sperm examination in a private laboratory located in the municipality of Cascavel, Paraná, from January 2014 to December 2016. **Results:** It was verified that of 888 tests analyzed, only 1% presented all parameters analyzed within normality, while 99% of the cases presented some type of alteration. There was a change in the following parameters with statistical significance between the number of reports analyzed: time of liquefaction, sperm concentration, head anomalies, tail abnormalities, number of spermatocytes and spermatids ($p < 0.0001$), viscosity ($p = 0.1098$), live forms ($p = 0.0524$), progressive motility ($p = 0.0010$) and total motility ($p = 0.0013$). **Conclusions:** There was a high prevalence of altered spermograms and a great number of alterations were observed, which requires the need for complementary tests to determine the causes of these alterations.

Keywords: semen; semen analysis; infertility.

Introduction

According to the American Society of Reproductive Medicine,[1] infertility is the disease of the reproductive system, characterized by the inability of a couple to bear children after one year of sexual intercourse without the use of contraceptive methods. Reproductive inability can be related to innumerable causes, such as anatomical, hormonal, genetic, biochemical and immunological changes, arising from infections, exposure to environmental factors or even the individual's lifestyle.[2]

Humans have moderately low fertility. Only 50% of couples with reproductive capacity will have children in three months, 75% in six months, 90% in one year and 95% in two years.[3-5] Male infertility affects on average 10% of men of age however, most of these cases have specific treatment.[6] This treatment, in turn, should be guided by professionals who can handle the needs of each patient.[7] The average is that 1 in 15 men have fertility problems.[8] According to Dohle et al.[9], research by the World Health Organization (WHO) shows that in every 4 couples seeking treatment for infertility, in at least one of them, both sexes have abnormalities. The inability to generate children reaches men and women around the world. In 2010, approximately 48.5% of couples had infertility problems. In addition, methodological differences and diagnostic errors greatly increase these numbers.[7]

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According to the epidemiological studies of Boivin et al.[4], in developed countries the incidence of infertility varies from 3.5% to 16.7% after one year of sexual intercourse without contraceptive methods. In the least developed countries, there was a change from 6.9% to 9.3%, with a global average of 9%. In Brazil, infertility affects on average 18% of couples, and the most common causes are episodes of pelvic infectious processes contracted by sexual intercourse and postpartum, or post-abortion due to precarious situations.[10] Thus, the present study aims to determine the prevalence and main changes observed in the semen of individuals living in the Cascavel municipality, in the west of Paraná, Brazil, from January 2014 to December 2016.

Methods

This is a cross-sectional and descriptive study, in which data were collected from sperm-type electronic reports in the SIAC system (Sistema Integrado de Análises Clínicas), issued by a private laboratory located in the municipality of Cascavel, Paraná, in the period from January 2014 to December 2016. The Cascavel municipality (24º 57′ 21″ S, 53º 27′ 19″ W), located in the west region of Paraná, has a land area of 2,100,831 m^2 and a population of 312,778 inhabitants, as estimated by the Instituto Brasileiro de Geografia e Estatística (IBGE).

All the reports of patients residing in Cascavel, who performed seminal analysis during the period determined in the laboratory in question, were included in the study, and reports were excluded from patients not resident in Cascavel, or who performed seminal analysis outside the research period.

According to the laboratory that carried out the analyzes, patients were instructed to maintain a abstinence time of two to five days and to perform semen collection by masturbation and to deposit in sterile bottles. After collection, the seminal fluid was maintained at a temperature of 37 °C until complete liquefaction and then macroscopic and microscopic examinations were performed. The methodology used for the sperm analysis was according to the World Health Organization.

The following variables were collected from the analyzed reports: service order number, initials of each patient, date of examination, liquefaction time, volume, appearance, color, viscosity, pH, sperm concentration, number of live forms, motility, morphology, type and percentage of anomalies (head, middle part and tail), number of leukocytes and red blood cells.

The data collected were stored in Microsoft Excel® and for analysis and study on the association between variables, the Chi-square test (test x^2) was used at a significance level of 5.0%, with results considered statistically significant for $p \leq 0.05$. Statistical analysis was performed using Bioestat® software, version 4.0. The work was approved by the Ethics Committee in Human Research beings of the University Center of the Assis Gurgacz Foundation, under opinion 857.540/2014.

Results

A total of 888 reports, of which 9 (1%), were within the normal parameters considered by the Laboratory Manual for the Examination and Processing of Human Sperm,[11] the remaining 879 (99%) presented results with some type of changes in the parameters evaluated. The volume, color, viscosity, pH, sperm concentration, number of live forms, motility, morphology, type and percentage of anomalies (head, middle part and tail), number of leucocytes and red blood cells. Regarding macroscopic examination, the majority of the individuals presented parameters for seminal analysis outside the values established by the World Health Organization. Viscosity changes were observed, with 39.3% (348) of the samples showing increased viscosity and 37.2% (330) decreased. Regarding the parameters volume, appearance and coloration, 15.7% (140) presented volume below that established, 90% (798) homogeneous appearance and 97.4% (865) pearl white coloration. In 80.6% (716) of the cases, the liquefaction time remained less than or equal to 60 minutes and in 89.0% (825) the pH was greater than or equal to 7.2 (Table 1).

In the microscopic analysis, 72.7% (587) of the reports had sperm concentrations within the established (≥ 15 million/ml) and 39.8% (305) showed vitality below the indicated values. In the analyzed cases, up to 1000 leukocytes/mm^3 and 98.4% (860) up to 1000 red cells/mm^3 were found in 98% (857), which is within normal limits (Table 2).

Regarding progressive motility, 67% (519) were below recommended and total motility, 66.6% (516) of the cases presented values below that established. The morphology was found to be within the standards in 99.9% (745) of the reports, and spermatocytes and spermatids were also within the limits in 73.3% (561) of the cases (Table 3). The data in Table 4 shows the types and percentage of the anomalies found. Among the head anomalies, microcephalic ones were the ones that presented the most changes (86.2%/658 cases). Two types of intermediate part anomalies were observed, the thickened being verified in a greater percentage of spermatozoa (95.4%/725). For tail abnormalities, rolled up was seen in most cases (96.2%/732).

Table 1. Macroscopic characteristics of the semen of individuals who performed sperm in a clinical analysis laboratory in Cascavel, Paraná, Brazil, 2014 - 2016

Variables Analyzed	Reference values	Reports		p-value
		n	%	
Volume	≥ 1.5 ml	748	84.2	<0.0001
	< 1.5 ml	140	15.7	
Aspect	Homogeneous	798	90.0	<0.0001
	Heterogeneous	89	10.0	
Color	Pearl White	865	97.4	<0.0001
	Yellow	16	1.8	
	Reddish	3	0.3	
	Brownish	4	0.4	
Liquefaction time	Until 60 min	716	80.6	<0.0001
	> 60 min	172	19.4	
Viscosity	Normal	208	23.5	0.098
	Increased	348	39.3	
	Decreased	330	37.2	
pH	≤ 7.0	36	4.1	<0.0001
	≥ 7.2	788	89.0	
	> 8.2	61	6.9	

Table 2. Spermatozoa number, live forms, leukocytes and erythrocytes in individuals who performed sperm in a private laboratory in Cascavel, Paraná, 2014 - 2016

Variables Analyzed	Reference value	Reports within normality	Reports out of normality	P-value
		n (%)	n (%)	
Sperm concentration	≥ 15 millions/mL	587 (72.7)	220 (27.3)	<0.0001
Living Forms (Vitality)	≥ 58%	461 (60.2)	305 (39.8)	0.0524
Leukocytes/ml	Until 1000/mm³	857 (98.0)	18 (2.0)	<0.0001
Blood cells/ml	Until 1000/mm³	860 (98.4)	14 (1.6)	<0.0001

Table 3. Progressive and total motility, normal spermatozoa, spermatocytes and spermatids in individuals who performed sperm in a private laboratory in Cascavel, PR, 2014 - 2016

Variables Analyzed	Reference value	Reports within normality	Reports out of normality	P-value
		n (%)	n (%)	
Progressive Motility	≥ 32%	256 (33.0)	519 (67.0)	0.0010
Total Motility	≥ 40%	259 (33.4)	516 (66.6)	0.0013
Normal spermatozoa	≥ 4%	745 (99.9)	1 (0.1)	<0.0001
Spermatocytes and spermatids	Until 4 in 100	561 (73.3)	204 (26.7)	<0.0001

Table 4. Anomalies of head, intermediate piece and tail in the reports analyzed in individuals who performed sperm in a private laboratory in Cascavel, Paraná, 2014 - 2016

Anomalies	Reports number with anomalies presence		P-value
	n	%	
Head anomalies			
Macrocephalic	619	80.9	
Microcephalic	658	86.2	
Fusiform	487	64.5	<0.0001
Bicephalic	285	37.7	
Amorphous	297	39.2	
Intermediate anomalies part			
Angled	683	89.9	0.7465
Thickened	725	95.3	
Tail anomalies			
Short	559	73.6	<0.0001
Curled up	732	96.2	
Double	316	41.5	

Discussion

Although age was not described in the reports, it is one of the important factors when talking about male infertility. Studies confirm that semen quality decreases after 40 years, leading to changes in ejaculate such as sperm movement, morphology, and decreased sperm concentration.[12] Regarding the microscopic examination, some parameters analyzed differed from those indicated by the World Health Organization. Health. In cases of motility, both progressive (67%) and total (66.6%) presented values below that specified. In a study reviewing WHO seminal parameters, they found that motility and concentration are the most correct factors to indicate fertilization capacity.[13] One study considered sperm motility to be the most important when compared to the other seminal parameters. When motility values are lower than those established, there may be a great difficulty in generating children.[14] In relation to sperm concentration, approximately 27.3% of the cases presented values below that stipulated. The reasons for sperm decline may be due to decreased sexual abstinence, or due to genetic disorders or infections.[15-17]

Among the agents that motivate changes in the quality of ejaculate are alcohol and tobacco that reduce sperm concentration and motility.[18] This study showed that in 39.8% of the cases the vitality was below 58%, which is considered reference value. A study carried out to evaluate the seminal parameters of 998 men, aged 20 to 60 years, observed that age is negatively associated with vitality, progressive motility and percentage of normal spermatozoa.[19] Several studies have found a negative relationship between obesity and seminal parameters, verifying a reduction in vitality, sperm concentration, morphology, motility and sperm DNA integrity.[20]

The least divergent of the patterns was the morphology (Kruger morphology), since normal values were verified in 99.9% of the cases. Some studies have investigated the relationship between semen morphology and age, and most of these studies show a reduction in the percentage of normal spermatozoa.[21-23]

When head and tail abnormalities were analyzed, statistically significant results ($p<0.0001$) were observed, but when the anomaly of the intermediate part was analyzed, a significant result was not observed. In the studies of Schwartz et al.[24], there was a small but significant decrease in morphological quality with advancing age. They also highlight an increase in the percentage of spermatozoa with head anomalies (microcephalus) and tail anomalies.

In the present study it was observed that leukocytes in 98% of cases remained within normal limits and only 2% of the cases presented indices below that established. In the research by Lackner et al.[25], the presence of pathogenic bacteria in asymptomatic men was observed in the absence of leukocytospermia. He also noted that low concentrations of leukocytes are detrimental to semen quality and fertility. In 39.3% of cases, increased viscosity was observed. The increased viscosity influences the liquefaction, which may be due to a reduction in the activity of prostatic enzymes,[26] in this way impairing semen quality.

Another significant parameter was the volume, which in 84.2% was within normal, being only 15.7% below that established. When Brahem et al.[27] analyzed the semen parameters of 140 fertile and infertile men aged 24 to 70 years, they found that both fertile and infertile men presented a reduction in volume (0.4 to 0.8 ml) in men with age greater than or equal to 50 years when confronted with men younger than 50 years. There is little information that does not link seminal volume to the age of man.[24,28-29] Some studies have observed a reduction in volume in young men, 21-35 years, and in older men (46-50 years), larger volumes are observed in men aged 26 to 45 years.[21,24,28]

Another parameter that presented significance was the liquefaction time, which should occur in up to 60 minutes to be considered complete. 19.4% of the cases presented incomplete liquefaction, since they liquefied after the 60 minutes. Incomplete liquefaction, after the set time of incubation, may indicate the lack of prostatic enzymes.[30] In the present study, 4.07% of the reports had a pH equal to or lower than 7. When it has a low concentration and a pH lower than 7, it can mean an ejaculatory canal block or a bilateral congenital lack of the vas deferens.[11,30]

The coloration of a homogeneous and liquefied sample is considered white, gray or yellow.[11] In the present study, it was observed that in 99.21% of the cases the color remained within normality. When talking about diagnosis of infertility, the sperm must be performed along with other tests to assist in the assessment. Several analyzes can be used to investigate a probable alteration, such as hormonal dosage, sperm analysis, karyotype analysis, histopathological examination of the testes[21,31-33], antibody search, sperm fragmentation test and scrotal ultrasonography.[34] These examinations, together with the spermogram, help in a more accurate diagnosis.

This study had some limitations. During the research, the absence of some information in the electronic reports was observed, minimizing the comparison of the information. In addition, our sample was a convenience sample, that is, the study was conducted only with those patients who sought care from a private laboratory, which may have been a source of bias, since these patients may present clinical, laboratory and epidemiological characteristics different from those seen in the public health system. In addition, we can observe differences when the sample is larger, that is, when patients from a larger number of laboratories are compared. Despite the inherent limitations of the manuscript, to a certain extent, it brings a positive contribution in relation to the epidemiological characteristics of the patients who presented spermogram alterations.

Conclusion

It was observed that 99% (879) of the analyzed reports presented some type of alteration in relation to semen quality. The parameters that had a greater statistical significance in relation to normal and altered were volume, appearance, color, time of liquefaction, pH, sperm concentration, leukocytes, red blood cells, normal spermatozoa, head anomalies, tail anomalies, spermatocytes and spermatids. It is necessary to research and carry out complementary tests in order to guide patients about the aetiology of the problems observed for the use of measures that may contribute to their reduction and their consequences in relation to the possibility of these men having children.

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DWR was responsible for data collection, design and writing of the manuscript. HSM was responsible for correction and translation of the manuscript. VLB was responsible for study design. CMS was responsible for revision and correction of the manuscript. DMI was responsible for data collection. JJVT was responsible for final revision and correction of the manuscript. LDP was responsible for study design, data analysis and interpretation.