Study of Variables Involved in Male Infertility Identified in the Spermograms Assessed in Assisted Human Reproduction

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ABSTRACT

Introduction: According to the World Health Organization, about 8 to 10% of couples worldwide have infertility problems and male internal aspects are the main reasons for half of occurrences of human sterility. Through the spermogram, it is possible to qualitatively and quantitatively analyze semen, contributing to the diagnosis of male fertile state. Objective: To study the relationship among sperm viscosity, concentration, motility and volume parameters and male infertility factors and to show the influence of the subject age on these seminal parameters. Methodology: A survey was conducted in the male infertility database of the Nascer Clinic (Recife / Pernambuco) of men aged 27 to 61 years, with a history of marital infertility, from 2018 to 2019. The subjects studied were grouped into categories according to the classification of the seminal parameters analyzed (volume, concentration, motility and viscosity) in their sperm. Student’s t-test was used for normal distribution and Mann-Whitney test for non-normal using the GraphPad Prism 8 program. Results: Among the studied individuals, there was a significant difference (p<0.05) between the populations with obstructive azoospermia and nonobstructive azoospermia and among the percentages of oligozoospermic individuals with obstructive azoospermia. The azoospermia group had a significantly higher average age than the normozoospermia group. Conclusions: This suggests that azoospermia is present in older men, compromising male fertility. Sperm testing should be performed by all men of childbearing age to investigate possible changes in the genesis of gametic cells.

Keywords: age groups, azoospermia, semen.

How to cite this article:
Bárbara Stefany da Silva Souza, Evandro Valentim da Silva, Sérgio Antônio Santos da Costa e Silva, Ana Maria Medeiros de Ataídes, Fálba Bernadete Ramos dos Anjos, Adriana Fracasso. Study of Variables Involved in Male Infertility Identified in the Spermograms Assessed in Assisted Human Reproduction. International Journal of Aging Research, 2020, 3:62
INTRODUCTION
Assisted Human Reproduction (RHA) consists of appropriate technologies for the study of human infertility through the manipulation of female and male gametes in laboratory and the subsequent introduction of these cells into the female reproductive system artificially. According to the World Health Organization (WHO), about 8 to 10% of couples worldwide, i.e., 50 to 80 million people, are experiencing some kind of infertility problem, with male internal aspects being the main reason for half of the occurrences of human sterility. The sperm count should be the most important element in the investigation of male infertility. To perform this exam, it is necessary to obtain a seminal sample that comes from a practical and noninvasive procedure: masturbation. Through it, it is possible to qualitatively and quantitatively analyze semen (liquefaction time, viscosity, turbidity, color, pH, concentration, motility, morphology, and vitality), contributing to the diagnosis of male fertile state. In opposite to what happens to women, the fertile potential of men is maintained practically throughout their lives. In this context, there are few studies in the Brazilian literature about the influence of male age on fertility. Some studies show that increasing age influences spermatogenesis negatively, so that the number of sperm formed from spermatogonia decreases gradually with male aging. The data described above justifies this study, since it is important to produce new studies to investigate the influence of male age on spermatogenic efficacy, enriching the Brazilian literature. Moreover, there is a close relationship between the intrinsic processes of primordial germ cell formation and characterization and the genetic and environmental events that occur during development, which possibly lead to infertility. Therefore, the spermogram is an essential tool in the process of investigating sperm status and possible changes in the genesis of gametic cells. The aim of this study was to analyze the relationship between sperm viscosity, concentration, motility, and volume parameters and male infertility factors, as well as to show the influence of age on these seminal parameters.

MATERIALS AND METHODS
Study population: We evaluated men from 27 to 61 years old who seek the clinic to undergo the RHA process, with a history of marital infertility (whether or not male), from 2018 to 2019. In this study it was not possible to analyze seminal parameters such as volume, motility, and viscosity of the azoospermia group, since individuals with this condition present total absence of sperm in the ejaculate. Therefore, only the sperm concentration of this group was considered and counted as 0 and for the other variables (motility, volume, and viscosity) azoospermic individuals were not included. Data collection: For this study, a survey was carried out on the infertility database of the Nascer Clinic (Recife / Pernambuco) of couples seeking RHA. The study of the quality and quantity of sperm collected by masturbation for the RHA procedure followed the criteria for the evaluation of normality of conventional seminal parameters according to the guidelines of the World Health Organization (WHO) (2010). At this stage we considered the patient's history, physical examination and seminal analysis obtained from stimulation. Statistical analysis: All data were expressed around the mean. The significance of the results was through analysis of variance, through the Prism Graphic Pad Statistical Program (GRAPHPAD, 2005). Statistical differences considered significant were p <0.05. Student's t-test was used for normal distribution or equivalent for non-parametric (Mann-Whitney test), used for non-normal distributions. To compare the average age of the normal group to the groups with alterations, the t test was used through the GraphPad Prism 8 program.

RESULTS AND DISCUSSION
Out of the 109 cases analyzed in the clinic from 2018 to 2019, 40.6% showed male infertility factors. These factors were used: an oligozoospermia (39.66%), followed by
asthenozoospermia (17.24 %) and obstructive azoospermia (18.96 %) (graphic 1).

Out of the last ones, 17.24 % of patients underwent Percutaneous Sperm Aspiration (PESA) and only 1.72 % underwent Testicular Sperm Aspiration (TESA). Moreover, oligoastenozoospermia represents 15.52 % of the cases, followed by 5.17 % of the cases that were classified as serodiscortant and 1.72 % that presented genetic problems of the altered karyotype. There were still 1.72 % of cases in which the cause of infertility was nonobstructive azoospermia. It is noteworthy that 4.9 % of the cases described infertility without apparent cause. It is estimated that men are responsible for 40 to 50 % of infertility cases, and woman 25 % and the remaining 20 % for idiopathic infertility. A possibility of almost equal infertility ratios is divided between undiscarded male and female factors, once the percentage of unexplained causes is excluded8,9. Some authors have commented that azoospermia is characterized by the complete absence of sperm in the ejaculate. An obstruction may occur in the ductal system, preventing the progress of sperm (obstructive azoospermia (AO) or reduced production of these cells10. Cases of azoospermia are present in the order of 1-2 % of the male population, being observed in 15-20 % in infertile men11. In the study population, of the 40.6 % of infertile men, 18.96 % showed obstructive azoospermia, while 1.72 % azoospermia is not obstructive, showing that they had some difference between those who used p <0.05. Obstructive azoospermia can be identified in men with cystic fibrosis, for example, due to congenital deferred vessel agenesis as a consequence of the Cystic Fibrosis Transmembrane Conductance Regulator (CFTR) gene and some mutation12. Among these patients, some of them may be carriers of this disease and may identify this disease. Cases of azoospermia do not obstruct related to a sperm disability (graph 1). This condition is characterized by the absence of these cells in the seminal ejaculate. In addition, the main morphological changes evidenced in the testis biopsy were applied to germ cells (11-20 %),
spermatogenesis maturation arrest (4 to 40 %) and hypospermatogenesis (50 %)\textsuperscript{12,13}. The percentage of infertile men with oligozoospermia was high compared to individuals with obstructive azoospermia (p <0.05). These abnormality parameters affect 50 % of men with infertility, being a more common alteration in oligoastenoteratozoospermia\textsuperscript{14}. As the genetic abnormalities found seem to be classified as less important when compared to an obstructive azoospermia (p <0.05), but it is relevant data. As genetic abnormalities, their involvement with male infertility and severe oligozoospermia and azoospermia increased from 10 to 12 % of the male population\textsuperscript{15,16,17}. The 109 selected in this study were grouped into age ranges. Most patients were aged 35-42 years, representing 45 % of the individuals studied. In 31.2 % of the cases, patients were 27-34 years old (Graph 2).

Graph 2 - Number of individuals assisted in Reproduction clinic, corresponding to each age group.

The average age of all men studied was 38.77 ± 6.63 years (range: 27 to 61 years). The mean volume of the seminal sample was 2.51 ± 1.04 mL (0.5 to 6 mL). The average concentration was 35.95 ± 44.76 million sperm per mL of semen (0 to 300 million / mL). The average progressive mobility (type A + B sperm) was 45.58 ± 19.09 % of the total sperm sample (Table 1).

Table 1 - Seminal parameters of the studied population.

| Variables                  | N   | Min | Max | Average | Median | Standard Deviation | Standard Error |
|---------------------------|-----|-----|-----|---------|--------|--------------------|----------------|
| Sperm concentration (x10^6/mL) | 109 | 0   | 300 | 35.95   | 20     | 44.76              | 4.29           |
| Motility A+B (%)           | 99  | 0.6 | 82  | 45.58   | 50     | 19.09              | 1.91           |
| Volume (mL)                | 99  | 0.5 | 6   | 2.51    | 2      | 1.04               | 0.10           |
| Age (years)                | 109 | 27  | 61  | 38.77   | 38     | 6.63               | 0.63           |

Source: Personal Collection.
Semen sample volume was normal in 87.88% of subjects; was below 1.5 mL in 9.09% and above 5 mL in 3.03% of the available samples. The study of sperm concentration revealed normozoospermia in 61.82% of the samples, azoospermia in 9.17%, oligozoospermia in 28.44%. A total of sperm with progressive mobility (types A + B) was normal in 79.8% of the packages, while the asthenozoospermic group represented 6.06% of these cases. In 93.94% of the cases normal viscosity patients and only 6.06% had increased viscosity (Table 2).

Table 2 - Influence of age on seminal parameters.

| Seminal Parameters | N   | % total | Average age | Standard Deviation | p       |
|--------------------|-----|---------|-------------|--------------------|---------|
| Volume (n= 99)     |     |         |             |                    |         |
| Hypospermia        | 9   | 9,09    | 34,5        | ± 5,6              | 0,1065  |
| Normospermia       | 87  | 87,88   | 37,9        | ± 5,8              | -       |
| Hyperspermia       | 3   | 3,03    | 43,7        | ± 2,1              | 0,093   |
| Concentration (n= 109) |     |         |             |                    |         |
| Azoospermia        | 10  | 9,17    | 47,1        | ± 8                | <0,0001 |
| Oligozoospermia    | 31  | 28,44   | 37,7        | ± 5,4              | 0,8841  |
| Normozoospermia    | 68  | 61,82   | 37,9        | ± 6,1              | -       |
| Progressive motility (n= 99) |     |         |             |                    |         |
| Normal             | 79  | 78,80   | 38,1        | ± 6,1              | -       |
| Astenozoospermia   | 20  | 20,20   | 37          | ± 5                | 0,4581  |
| Viscosity (n= 99)  |     |         |             |                    |         |
| Normal             | 93  | 93,94   | 37,8        | ± 6                | -       |
| Increased          | 6   | 6,06    | 40,2        | ± 2,6              | 0,3401  |

Source: Personal Collection.

Research shows a reduction in seminal quality in recent years associated with aging and other factors such as alcoholism, smoking or even drug use\(^\text{18-21}\). And this drop also appears in fertile and young individuals, presenting reduction in sperm concentration, seminal volume, motility and increase of abnormal sperm morphologies\(^\text{22,23}\). A recent review has found it difficult to find in the literature a clear relationship between increased paternal age and sperm concentration\(^\text{24}\). Azoospermic individuals were older than normozoospermic ones, however, when compared, the average ages did not present significant differences\(^\text{25}\). However, in the present analysis, the azoospermia group had a significantly higher average age than the normozoospermia group, suggesting a decrease in concentration with increasing age. Some studies have found this same results\(^\text{26,27}\) while others have revealed opposite effects, showing an increase in sperm concentration with increasing male age\(^\text{6,28,29}\). Most studies in the literature agree that sperm volume decreases with increasing male age\(^\text{24}\), revealing\(^\text{28}\) a decrease in seminal volume (0.15–0.5 %) with each year of added age. In contrast, this survey is not present in this study, since no significant differences were found between the mean age and hypopermia. Patients with hyperpermia had higher mean ages than normosperms, but it was
not significant. Progressive motility can influence pregnancy rates and, therefore, is a parameter used to check seminal quality\textsuperscript{30}. The relationship between increasing age and decreasing progressive sperm motility is well evidenced in different studies. One of them demonstrated that, with each year of increased age, there is a decrease of about 3.1 \% in progressive motility in healthy individuals\textsuperscript{21,24}. Most of the studies cited above were based on the 1999 WHO manual, which is outdated as to the required seminal parameter values. In the present study we used the WHO 2010 manual, with the latest values, providing a correct and current analysis of the influence of age on semen quality.

CONCLUSIONS

Male infertility may be related to genetic and environmental factors that possibly interfere with the quality of life of couples seeking Assisted Human Reproduction assistance. The spermogram is an important tool for sperm diagnosis. This study suggests that azoospermia is present in older men, compromising male fertility. Therefore, sperm should be performed by all men of childbearing age in order to investigate possible biochemical and biophysical lesions or responses in the genesis of gametic cells.

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