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Original paper

Influence of age on sperm parameters in men with suspected infertility

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Abstract

Male fertility has become a very discussed topic among researchers in the last years. With the latest environmental changes, use of genetically modified foods, lack of physical exercise, work overload and frequent burnouts, the number of men prone to infertility is continuously increasing. In this study, we aimed to evaluate the influence of age on the sperm quality in presumably infertile males. In this purpose, we have investigated a significant number of semen samples, by using macroscopic and microscopic standard evaluation methods to determine the volume, viscosity, density, morphology, motility and vitality of spermatozoa. Our data suggest that spermatozoa viability and progressive motility, two of the most important indicators of sperm quality, decrease with age, suggesting the role of patient's age in male infertility, as well as the necessity for a better understanding of the intimate mechanisms and risks involved, as an increasing number of men are choosing to delay fatherhood.

Keywords : infertility, male population, age threshold, sperm parameters, motility, density, spermatozoa

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Introduction

Male infertility is defined as failure to produce spermatozoa of high quality, in terms of density, morphology or motility (VOGT 2004, [1]; W.H.O 2010, [2]). The term "male infertility" does not have a well-defined clinical concurrence, but rather constitutes a collection of different clinical conditions with a variety of possible etiologies and prognosis. (AITKEN et al. 1995, [3]; PASQUALOTTO et al. 2011, [4]). Factors such as urogenital infections, exposure to toxic substances and vascular diseases may impair seminal quality, resulting in embryonic defects, loss of pregnancy or children highly prone to develop genetic diseases and other pathologies (CAVALCANTE et al. 2008, [5]). The correct estimation of male fertility potential has become of great interest to researchers, but available tests are not always accurate for this specific pathology. On the other hand, when the results of these tests are under the normal values, they do not clearly state the diagnosis of sterility. Sperm analysis is one of the basic recommendations for the initial investigation in infertile couples. Since its first publication in 1980, the WHO manual used in andrology laboratories (with four updated editions, the last one published in 2010) recommends the standard methodology for human semen analysis (W.H.O. 2010, [2]). The first studies made to correlate the quality of seminal parameters with male fertility date back to 1930s (MACOMBER & SANDERS 1929, [6]). Although the analysis of seminal samples provides valuable information about male fertility, it is not always sufficient to determine the exact cause of infertility (WANG & SWERDLOFF 2014, [7]).

Materials and Methods

The current study was conducted over a period of 5 years (April 2013 - April 2017) on 500 semen samples from ambulatory care patients investigated for infertility. The samples were examined both macroscopically (to measure the volume, viscosity, appearance, odor and pH) and microscopically, to evaluate the presence of mucus filaments, aggregation or agglutination of spermatozoa (i) type A: "head-to-head"; ii) type B: "tail-to-tail"; iii) type C: through the tips of the tail; iv) type D: mixed - "head-to-head" and "tail-to-tail"; and v) type E agglutination: queue agglutination, but with non-free heads present in the agglutinate); to determine the presence of other cells: epithelial, immature germ cells, leukocytes, isolated sperm heads and ends; to assess sperm motility (i) "A"- fast, with beatings of the tail in the narrow beam, accompanied by lateral head movements and straight line progression; ii) "B" - slow, with head movements, but without tail beatings and with or without rectilinear motility; iii) "C" - low mobility, with disordered vibrations, frenzy, tail rotation and no movement; iv) "D" - immobile sperm, without tail movements, that can sometimes be confused with dead spermatozoa); to establish density, vitality and total number of spermatozoa (through eosin - nigrosin staining); to establish sperm morphology (through the inventory of head, intermediate piece and tail defects); to count white blood cells (C-Chip camera/Endtz Method).

According to the WHO criteria established in 2010, presented in Table 1, the seminal parameters values must fall within standard reference range, although some authors discuss in their papers about the drawbacks of using a single threshold value to distinguish the normal and abnormal values of seminal parameters (Patel et al. 2018, [8]).

Table 1. Semen parameters reference ranges defined by WHO, 2010 edittion.

Parameters	Reference range
Semen volume (mL)	1,5 (1,4-1,7)
Total number of spermatozoa (10^6/ejaculate)	39 (33-46)
Spermatozoa density (10^6/mL)	15 (12-16)
Total motility (PR + NP)	40 (38-42)
Progressive motility (PR)	32 (31-34)
Vitality (live spermatozoa, %)	58 (55-63)
Sperm morphology (normal, %)	4 (3-4)
Other threshold values	
pH	>7.2
Peroxidase positive leukocytes (10^6/mL)	<1

Legend: PR - Progressive motility; NP - Non-progressive motility; MAR - Mixed antiglobulin reaction

Results and Discussions

During the timeframe of the present study, it was possible to observe the increasing trend in the number of men who requested an investigation of their seminal parameters during. Thus, if 5% of the patients enrolled in this study were monitored in 2013, their number increased with time reaching 19% in 2014, 20-26% in 2016, growing to 29% in 2016 and ultimately reaching 21% in the first 8 months of 2017.

The patients enrolled in this study were distributed according to similar studies into 6 age groups, in order to be able to perform a comparative interpretation of the obtained results, i.e.: 18-25; 26-30; 31-35; 36-40; 41-48 years old; > 49 years. The age group of 31-35 years included the highest number of patients (165) (33%), followed by the group of 36-40 years (130) and ultimately by the 41-48 years old age group (118).

The age mean of the patients enrolled in this cohort study was 34.61 ± 5.85 years. Similar results were reported by CASTRO et al. 2012, [9] by analyzing a population sample with an average age of 32.47 ± 8.39 years. FARIA et al. 2012, [10] reported that the age of patients with infertility problems was ranging from 24 to 54-year-old.

From studying the quality of the sperm obtained from the 500 patients, we have recorded changes in all seminal parameters (Figure 1). It was noted that the most common abnormality was the sperm viability primarily found in 328 patients (66%), followed by sperm motility (with significant differences between progressive and total motility). In the case of progressive motility, 312 patients (63%) exhibited values inferior to the WHO normal range; whilst in the case of total motility, 214 patients (43%) presented lower values.

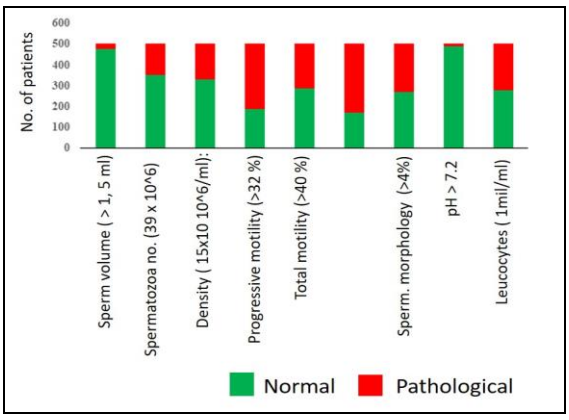

Figure 1. Distribution of normal and pathological values of different seminal parameters in the 500 investigated patients.

Similar results were obtained by Gao et al. 2008, [11] and Ramzan et al. 2015, [12] in a study performed on a population sample in China, uncovering abnornmal sperm viability in 73.8% of the analyzed samples. In the same group, it was found that 232 patients (50%) presented abnormal sperm morphology and leukocyte counts values.

In our study, the majority (90%) of the investigated patients had normal values of their semen volume. A volume below 1 mL of sperm indicates hypospermia, suggesting exposure to infections, retrograde ejaculation, congenital bilateral absence of the deferent vessels, poorly developed seminal vesicles, or an idiopathic origin (DOROFTEI et al. 2015, [13]).

This result is similar to the ones obtained from studies conducted in different regions of the world, suggesting that the volume mean of seminal material obtained from infertile and fertile subjects did not differ significantly (FISCH et al. 1996, [14]; HARRAWAY et al. 2000, [15]; JORGENSEN et al. 2001, [16]; AHMED et al. 2009, [17]; KHAN et al. 2013, [18]; RAMZAN et al. 2015, [12]).

The pH of seminal material was within normal range in most of the analyzed patients, except for a total of 11 males that presented a pH of less than 7.2. The changes in the pH value suggest anomalies of the auxiliary sexual glands or obstruction of the ejaculatory duct (DOROFTEI et al. 2015, [13]). The current study does not reveal significant differences in the pH values between subjects with normal/abnormal seminal parameters. Similar results were reported in studies performed on USA (Harraway et al. 2000, [15]), Norway (HAUGEN and GROTMOL 1998, [19]) and the Pakistani population (KHAN et al. 2011, [20]; RAMZAN et al. 2015, [12]).

Male infertility is multifactorial (AGARWAL et al. 2010, [21]) and any changes in the normal physiology of the reproductive organs can easily influence sperm functions and can lead to oligospermia (low sperm count), asthenozoospermia (reduced sperm motility), teratospermia (abnormal morphology of the spermatozoa), azoospermia (lack of sperm in the ejaculate) and, in some cases it can lead to oligoasthenoteratozoospermia (OAT), which is a major problem for successful fertilization (WHO 2010, [2]). In addition to many conventional causes, age plays an important role in the sperm physiology. Unlike women, men have the advantage of contributing to

conception at delayed ages (AMANN et al. 2008, [22]). However, starting with their 30's, degenerative changes of the seminal epithelium can be seen, and the decrease of the Leydig cell number and their function can lead to a reduced spermatogenesis through lower testosterone levels (WANG et al. 1993, [23]; JOHNSON 1986, [24]; HARMAN et al. 2001, [25]; AMARAL et al. 2009, [26]). Animal studies have shown that when the aging process starts, males produce sperm of lower quality (ESKENAZI et al. 2003, [27]). Age is correlated with a decrease in nitric oxide, which is associated with erectile dysfunction in older men. Moreover, with age, sperm quality decreases and a decline in the number of fertilized embryos is seen (DAIN et al. 2011, [28]; KIDD et al. 2001, [29]).

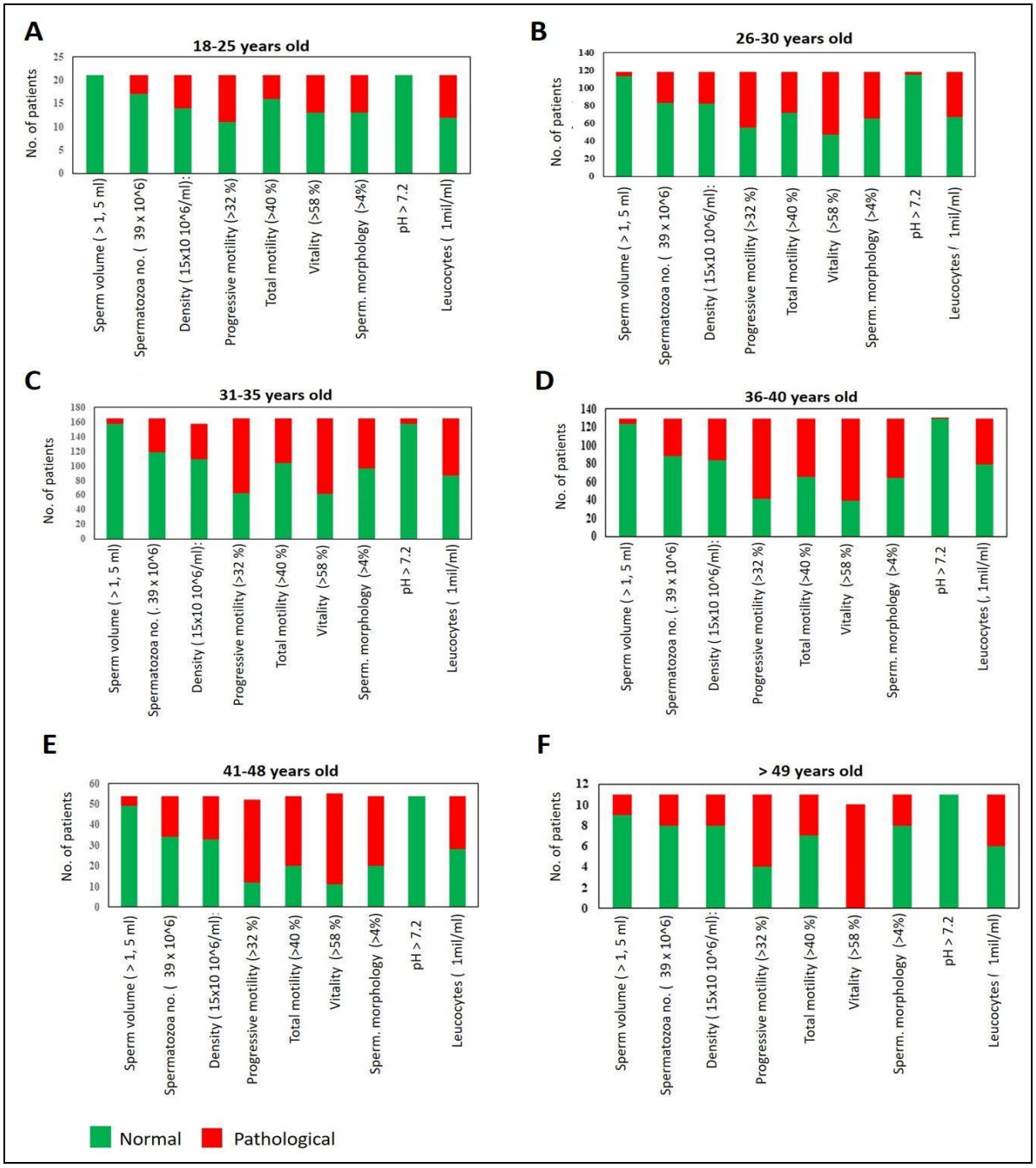

Figure 2. Distribution of patients belonging to different age groups, depending on the normal / pathological values of seminal parameters

Testicular volume and histopathological changes are strongly associated with age, resulting in hormonal changes. However, the age limit for sperm production is not defined (MAHOUD et al. 2003, [30]; SAMPSON et al. 2007, [31]). Moreover, delays of resorting to a better lifestyle can lead to the need for assisted reproduction (ZOFNAT et al. 2012, [32]).

As stated above, the males selected for this study were divided into six age groups. In the first group of 18-25 years old males, over 50% of patients had normal values for all seminal parameters (total sperm count/ejaculate - 17 patients, density -14 patients, progressive motility - 11 patients, total motility - 16 patients, sperm viability - 13 patients, sperm morphology - 13 patients and leukocyte count - 12 patients); as for two parameters (sperm volume and pH) - all 21 patients were within the normal range (Figure 2A).

Semen was collected from 118 patients within the age group of 26-30 years. After analysing the data, it was observed that over 96% of these patients had normal values of their sperm pH and over 55% of them also presented normal values for other seminal parameters (total sperm count/ejaculation - 83 patients, density - 82 patients, total motility - 72 patients, sperm morphology - 65 patients and leukocyte count - 67 patients). In the case of progressive motility, 55 patients (47%) presented normal values, while 47 patients (40%) had normal values of sperm viability (Figure 2B).

When compared with the first group of patients (ages from 18 to 25 years), it was observed that in the 26-30 years age range, over 50% of individuals present values below the WHO reference values for two seminal parameters (sperm viability - 55 cases and progressive motility of the spermatozoa - 47 patients).

A number of 165 males within the 31-35 years old age group had normal values of sperm volume and pH, meaning that they were within the normal range in over 96% of patients (Figure 2 C). The other seminal parameters were within normal range in over 50% of patients (72% - total sperm count, 66% sperm density, 63% total motility, 58% sperm morphology, 53% white blood cell count). When it comes to progressive motility and mobility, only 41% and 38% of cases had normal values.

Comparing these results with those obtained from the first two age categories, the decrease in progressive motility (50% in the 18-25 age group) and sperm viability (38% in the group of 31-35 years) is higher. All other seminal parameters showed similar values for the three age stages described in this study.

A number of 130 patients were included in the 36-40 years old age category. In Figure 2D, it can be noted that, as in the previous cases, sperm volume and pH values are within normal limits in over 95% of patients. The other seminal parameters turned out to have normal values in over 50% of patients (68% - total sperm count, 65% - sperm density, 51% total mobility, 50% sperm morphology, 60% leukocyte count). When it comes to progressive motility and viability, a decrease in their normal values was found in 25% and respectively 23% of men.

Semen samples were collected from 54 patients belonging to the age group of 41-48 years. Figure 2E shows normal values of their pH and volume in over 95% of cases. Also, three semen parameters were shown to have normal values in over 50% of patients, as follows: 63% - total sperm count; 61% - sperm density; 52% - the number of leukocytes; while four other seminal parameters presented abnormal values in over 50% of patients: 74% - progressive motility; 63% - total motility; 81% - sperm viability; 63% - normal sperm morphology. It can therefore be stated that with age, seminal parameters and sperm quality are significantly diminished. If the first two age categories (26-30 and 31-35 years old) showed only a slight decrease in viability and progressive motility, in the case of the 41-48 age group, there can be seen a much more pronounced decrease of the same seminal parameters (progressive motility and viability), plus a decrease in the total motility and sperm normal morphology.

In the age category of over 49 years, 11 patients presented for semen collection. Figure 2F shows that the values for pH and seminal volume are within the normal range in over 80% of the investigated men. Other seminal parameters presented normal values in over 50% of cases, as follows: 81% - total sperm count; 81% - semen density; 64% - total mobility; 81% - sperm morphology; 55% - the number of leukocytes. There are some exceptions, represented by progressive motility (although, 37% of patients are in the normal range) and viability, where all 11 patients present pathological values.

There are many studies that focus on the correlation between age and male / female infertility, all of them concluding that advanced paternal age is a strong risk factor of disorders that include production and sperm quality (CAVALCANTE et al. 2008, [5]).

Although there is no age limit for male fertility, studies reveal that fertility starts to decline at the age of 40 (TELÕKEN et al. 1999, [33]). Other studies indicate a decrease in semen volume in men after 40-50 years. In addition, some researchers demonstrate a reduction in progressive motility and normal sperm morphology with advanced age. On the other hand, information on the correlation between sperm density and aging is disputable (TELÕKEN et al. 1999, [33]; SILVA et al. 2012, [34]).

CASTRO et al. 2012, [9] compared the volume, progressive motility, normal morphology and sperm density in patients below/over 40 years of age. The authors found a statistically significant decrease in semen volume in patients over 40 years, while for the other parameters there were no significant differences present between these two age groups. Aging was also correlated with a decrease in sperm density and motility. On the other hand, a Brazilian retrospective study showed that sperm volume was the only parameter that diminishes with age, which corresponds to our findings. In our study the seminal volume was below the normal range in 11.2% of subjects, while other studies report abnormal values of this specific parameter in 23.4% of patients (CAVALCANTE et al. 2008, [5]).

Contradictory results that were reported in different studies may be due to country-specific cultural, environmental, economic and social factors, thus influencing the epidemiology of infertility (ELZANATY et al. 2005, [35]). It has been described that infertility patterns in developing countries differ from those in developed countries (BLACKWELL & ZANEVELD 1992, [36]).

Some studies have shown that with the growth of paternal age, sperm morphology deviates from normal (WALTER et al. 1998, [37]; ESKENAZI et al. 2003, [27]). ZHU et al. 2011, [38] performed sperm analysis in men, ranging between 20 and 60 years, showing that age has a negative effect on sperm morphology. Similar results have been reported by Kidd et al. 2001, [29] which demonstrated that normal sperm morphology decreases in 50-year-old patients compared to those that fit in the 30 years age category (WALTER et al. 1998, [37]).

Other studies have shown a decline in sperm viability and progressive motility starting with the group age of 31-35 years; lower values of these parameters being obtained as the subjects age (SUNANDA et al. 2014, [39]).

Similar results were obtained in a study performed on a population cohort in China by ZHU et al. 2011, [38] and Kuhnert et al. 2004, [40] who observed the downward trend in sperm viability, morphology and volume. Studies on population cohorts from Cordoba (Argentina) and the Arabian Gulf reported an age-related decrease in all seminal parameters (MOLINA et al. 2010, [41]; OMRAN et al. 2013, [42]). MUKHOPADHYAY et al. 2010, [21] have obtained similar results for motility, but not for sperm density. Since normal spermatozoa morphology is essential to ensure motility during sperm transit, it may be difficult to achieve a higher degree of motility, imperative for conception in older men. The decay of healthy germ cells in older men may be one of the reasons for motility loss (ESKENAZI et al. 2003, [27]). In our study, there was no significant change in semen volume, in contrast to another studies performed on elderly patients who switched to IVF and ICSI, and who had changes in their semen volume (DAIN et al. 2011, [28]).

Studies performed on laboratory animals have revealed histological changes of testes, seminiferous epithelium, germ cell defects, failure of preimplantation, and increased frequency of mutations in sperm DNA as men grow older. DNA damage was also more common in older men, leading to spontaneous abortion after IVF (KIDD et al. 2001, [25]).

ZHU et al. 2011, [38] presented a low reference value for motility (40.6%) and vitality in their paper (36.4%). But in the current study, the percentage of subjects with motility and viability parameters below the normal ranges established by WHO is very high in the case of total (50.8%) and progressive motility (43.4%). Good quality related semen was identified in patients from the ages of 20 until 34 years.

Since the entire spermatogenesis process is regulated by the Leydig-hypothalamus-pituitary gland and the androgen-dependent hormone secretions, continuous exposure to environmental toxic substances and today's lifestyle may be responsible for the alternations in the number and normal function of germ cells, Sertoli cells and Leydig cells, and this can explain the changes suffered by the reproductive system with age (CREASY 2001, [43]).

Conclusions

In conclusion, the results obtained from the evaluation of the 500 patients included in this study, distributed in different age groups, reveal that the sperm volume and pH are not influenced by age, these values being in the normal range in over 90% of patients, irrespective too their age group. In exchange, sperm viability and progressive motility decrease with age, targeting the group age of 31-35 years and over. The other seminal parameters (total sperm count, sperm density, total motility, sperm morphology, white blood cell count) fall below the normal range in approximately 50% of patients, being unrelated to any age group.

Conflict of interest disclosure

There are no known conflicts of interest in the publication of this article. The manuscript was read and approved by all authors.

Compliance with ethical standards

Any aspect of the work covered in this manuscript has been conducted with the ethical approval of all relevant bodies and that such approvals are acknowledged within the manuscript.

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